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Mating behavior and time budget of an androdioecious crustacean, *Eulimnadia texana* (Crustacea: Conchostraca)

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Key words: androdioecious crustacean, *Eulimnadia texana*, behavior, mating

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Abstract

The clam shrimp, *Eulimnadia texana* (Crustacea, Conchostraca), is found in freshwater ephemeral environments throughout the United States. Individual clam shrimp of this species are either hermaphroditic or male, a relatively rare mating system for animals known as androdioecy. Comparison of sex ratios between four neighboring populations of *E. texana* in Southern New Mexico showed wide variation in the ratio of males to hermaphrodites with males making up as much as 42% of some populations and not occurring at all within others. Since little is known about the behavior of this species, an ethogram and time budget were prepared based on observations of laboratory populations. Males attempt to clasp hermaphrodites prior to mating. Precopulatory mate guarding occurs in this species. Outcrossing generally occurs during mate guarding and after the hermaphrodite molts. Hermaphrodites, however, seem to control the mating process. Successful mating by males never occurred if the hermaphrodite struggled with him; hermaphrodites will self in the presence of males.

Introduction

The clam shrimp, *Eulimnadia texana* (Crustacea: Conchostraca) is found across North America (Sassaman, 1989). This species inhabits small ephemeral ponds that typically appear due to spring and summer rains. The puddles dry up and the eggs remain dormant until the puddles reform. *Eulimnadia texana* is androdioecious, a rare situation in animals in which a species has both male and hermaphroditic individuals (Sassaman & Weeks, 1993). Hermaphrodites can self or outcross with a male, but they cannot exchange sperm with other hermaphrodites (Sassaman & Weeks, 1993). The body of the clam shrimp is encased within a translucent, carapace through which most organs are visible. Clam shrimp have numerous pairs of legs which are used for feeding. *Eulimnadia texana* feeds primarily on algae though it will eat other types of detritus when algae is limited (Medland, 1989).

Pronounced sexual dimorphism allows easy identification of males and hermaphrodites. In the males, the first two pairs of thoracic appendages have been modified into claspers for grasping the hermaphrodite during outcrossing. Absence of such an adaptation pre-

vents hermaphrodites from outcrossing with one another (Sassaman & Weeks, 1993). The hermaphrodite's unfertilized eggs lie in paired ovaries along its digestive tract and are visible under a microscope. When fertilization occurs, the eggs move into a brood chamber where they are contained in a mucous mass between the hermaphrodite's dorsum and the carapace. The eggs are visible to the naked eye and aid in sexing the clam shrimp.

Research involving this species has focused primarily on the genetics and theoretical implications of this mating system (see Strenth, 1977; Sassaman, 1989; Sassaman & Weeks, 1993; Otto *et al.*, 1993). Sassaman & Weeks (1993) have shown that sex determination occurs at a single autosomal locus and that individuals are either monogenic hermaphrodites (those which produce only hermaphroditic offspring), amphigenic hermaphrodites (those which produce both male and hermaphroditic offspring), or male. Monogenic individuals are homozygous dominant at the autosomal locus, amphigenic individuals are heterozygous, and males are homozygous recessive (Sassaman & Weeks, 1993). This system is very similar to the reproductive systems of many Notostracans (Sassaman, 1991). Due

to the nature of sex determination, populations typically consist of many more hermaphrodites than males (Sassaman, 1989; pers. obs.).

Behavioral information on *E. texana* is largely unavailable in the primary literature and would be a useful supplement to ongoing evolutionary and genetic studies which are examining the significance of sex, inbreeding and outcrossing. To that end, this contribution provides an account of male and hermaphrodite behavior in the form of an ethogram, time budget and detailed descriptions of mating behavior.

Methods

Study populations

Four populations of *E. texana* have been identified just northeast of the Doña Ana Mountains, 40 km NNE of Las Cruces, Doña Ana County, New Mexico. These populations are all located within 4 km of one another. Three of the populations are located along the access road to the New Mexico State University College Ranch, next to South Well. These populations are referred to as SWP3, SWP4 and SWP5 (South Well puddle populations). Each of these sites consists of a distinct depression less than 10 meters in length and with a depth no greater than 1 meter at the lowest point. SWP5 is almost directly across a dirt road, 35 m from SWP3. SWP4 is 130 m west of SWP3 on the same side of the road. There is sparse vegetation immediately around these puddles predominantly consisting of various grass species. There is little to no vegetation within the puddles themselves. These sites typically fill with water at least once every year as a result of summer rainfall and remain filled for three days to several weeks depending upon further water inputs (pers. obs.). Other than *E. texana*, these sites are also occupied by populations of *Scaphiopus couchii* tadpoles, which hatch well after the clam shrimp mature (and thus do not present a severe predation problem) and are also used as watering holes by cattle (pers. obs.).

The fourth population occurs within the playa portion of the NSF Long-Term Ecological Research site on the New Mexico State University College Ranch (LTER Playa), approximately 4 km west of the other three sites. The playa encompasses a 12-ha basin (Creusere & Whitford, 1976). The LTER Playa has considerably more vegetation than the SWP populations, including *Prosopis*, *Panicum*, *Amaranthus*, *Hymenoxis*, and *Helianthus* (MacKay *et al.*, 1990).

The ecology and population dynamics of this site have been studied quite extensively (see Creusere & Whitford, 1976; Medland, 1989; VanVactor, 1989; MacKay *et al.*, 1990 for an overview). This site receives an average rainfall of 210 mm annually (MacKay *et al.*, 1990). The majority of this precipitation (55%) is attributed to convectional rainstorms during the late summer months (VanVactor, 1989; MacKay *et al.*, 1990). The site is relatively level and does not flood as often as the SWP populations, typically flooding once every three to ten years and remaining flooded for several weeks (MacKay *et al.*, 1990). In addition to *E. texana*, MacKay *et al.*, (1990), indicate that this site is also inhabited by the tadpole shrimp, *Triops longicaudatus*, and fairy shrimp species, *Thamnocephalus platyurus* and *Streptocephalus texanus* (D. Belk indicates that the report of *S. texanus* populations within the LTER Playa is likely a misidentification of a mixed population of *S. mackini* and *S. dorotheae*). The life cycles of these species all overlap (MacKay *et al.*, 1990). Cattle have not been allowed within the site since 1972 (MacKay *et al.*, 1990).

General sampling and rearing methods

Numerous samples of the first 1–2 cm of soil were collected from each study site; soil within each site was mixed to ensure randomness. Sample populations were established in the laboratory by placing 250 ml soil samples in plastic mouse cages (30 cm × 12 cm × 15 cm) and hydrating them with 4 liters of aged tap water. The tanks were kept on a 24 hour light cycle using incandescent bulbs in reflectors to maintain summer-like temperatures of approximately 30 °C and were supplemented with tadpole food pellets until algal growth was initiated. Clam shrimp hatched within approximately 24 hours of wetting. Tadpole shrimp, *Triops longicaudatus*, also hatched from the same soil samples and were removed from the tanks as soon as possible to prevent predation.

Sex ratio calculations

Sex ratios were calculated for all four populations. Ten tanks were set up from each population (40 tanks total) using the methods just outlined. Sex was determined for every individual hatching within a tank. Sex ratios were computed following the procedures outlined in Sassaman (1989) as soon as sexual dimorphism was apparent (approximately three days after hatching). Comparisons of total viable eggs per 250 ml soil sam-

ples were made between populations. The number of eggs initially contained within samples is unknown.

Ethogram

Initial observations of behavior were compiled into an ethogram of clam shrimp behavior following the guidelines outlined in Martin & Bateson (1993).

Clam shrimp time budget

The LTER Playa population was the only population used for the remainder of the study due to the availability of information on population dynamics for this site under field conditions. Individual LTER Playa clam shrimp were uniquely marked using a small dot of Testers enamel paint. Clam shrimp molt from the inside of the carapace so paint marks are not lost with subsequent molts and provide a fairly permanent means of identification. Though clam shrimp can be sexed within three days of hatching, they cannot be marked for individual identification until five days after hatching when the carapace no longer collapses when paint is applied.

Sample groups were established in the laboratory and housed in plastic mouse cages. The bottoms of the cages were lined with 1 cm of 'clean' sand (*i.e.*, sand free from Branchiopod eggs). Groups were composed of seven hermaphrodites and three males to reflect natural population sex ratios (see Results). The tanks were monitored using instantaneous scan sampling techniques as described by Altmann (1974). During each scan, the behavior of each clam shrimp was recorded during that moment in time. The order in which the clam shrimp were scanned was determined haphazardly based upon the order in which individuals came into view. Each tank was scanned six times a day at one hour intervals until less than half of the individuals (4 clam shrimp) remained within the sample population. Clam shrimp that died within the first two days of scanning were replaced with clam shrimp of the same sex and hatch date. Three control tanks were set up with the same sex ratios as the sample tanks but with minimal handling of the clam shrimp and no paint marking to determine whether handling and marking techniques had an adverse effect on life span.

Mating behavior

Other behavioral observations of *E. texana* were conducted using the focal animal technique outlined

Table 1. Sex ratios and number of viable eggs hatching within four populations of an androdioecious clam shrimp *Eulimnadia texana* from 250 ml soil samples.

Population	# of tanks	$\bar{x} \pm s.d.$ clam shrimp	Total # of ind. hatched	Percentages herm.: males
LTER	10	17 ± 7.2	170	70:30
SWP3	10	37 ± 8.8	374	100:0
SWP4	10	34 ± 5.0	342	60:40
SWP5	10	30 ± 7.6	328	58:42

by Altmann (1974). Groups consisting of either a male and a non-gravid hermaphrodite, two males and a non-gravid hermaphrodite, or two non-gravid hermaphrodites and a male, were collected from rearing tanks and isolated in a plastic 'dixie' cup containing 125 ml of aged tap water. Pairs were observed using focal animal observation techniques for two hours and all interactions were recorded. If the two hour observation was complete and either outcrossing or selfing appeared to be eminent, the observation was extended to include observation of egg fertilization. No observation ever lasted longer than 2 hours and 25 minutes. Each focal animal was only used once.

Analysis

Data are presented as means (\pm sd). For statistical procedures, an α level <0.05 is considered significant throughout this study.

Results

Sex ratios

The sex ratios for the four populations are presented in Table 1. In SWP4, SWP5, and the LTER population, sex ratios were skewed in favor of hermaphrodites. No males were ever found in the SWP3 population. Table 1 also provides the average number of clam shrimp hatching from a 250 ml soil sample for each population. There is no significant difference in total viable eggs hatching between the SWP3, SWP4 and SWP5 populations ($\chi^2 = 3.19$, $df = 2$, $P > 0.1$). The LTER Playa population had a significantly lower number of eggs hatch, however, when compared to the three puddle populations ($\chi^2 = 81.9$, $df = 3$, $P < 0.001$).

Ethogram

The major types of behaviors observed in initial observations are listed and described in Table 2. Behavioral categories have been broken down into those common to both reproductive forms, those specific to hermaphrodites, and those specific to males.

Clam shrimp time budget

Tanks were scanned over a period of 11 days for a total of 7 hours and 33 minutes of observations. Time budgets were computed for both males ($N=12$) and hermaphrodites ($N=28$) using the methods outlined in Martin & Bateson (1993). These results are summarized in Fig. 1. No distinction was made between feeding and swimming behaviors because the two occur simultaneously. These activities were the primary behaviors for both sexual forms.

The reproductive status of the hermaphrodites was recorded during each scan. Hermaphrodites were gravid 72% of the observation time. Hermaphrodites appear to dig burrows and may drop their clutches underground. On several occasions, males were seen entering burrows, but I never saw males digging their own. In six instances, males were seen clasping gravid hermaphrodites just prior to egg laying. This may be a form of pre-mate guarding.

Mortality rates were calculated for both the scan tanks and the control tanks. There was no difference in mortality rates between the two tank types ($\chi^2=1.28$, $df=1$, $P>0.2$). Pooling all tanks, male mortality was higher than that of hermaphrodites ($\chi^2=4.51$, $df=1$, $P<0.05$), especially starting on day 8 of life (Fig. 2). On average, hermaphrodites lived 50% longer than males. Each population was initially set up with a 3:7 ratio of males to hermaphrodites. By the end of the observations, there was a 1:4 ratio in each of the scan tanks. Pennak (1989) reported that Eubranchiopoda males typically die within an hour after outcrossing. This was not true for *E. texana* and probably not for other Eubranchiopoda as well (Belk, pers. comm.).

Clam shrimp mating behavior

During focal animal studies ($N=178$ groups), outcrossing occurred 95 times (53%), selfing occurred 20 times (11 %) and no fertilization was evident either due to selfing or to outcrossing in 63 of the observations (35%) (Fig. 3). In 46 of these cases, no contact was seen between the male and the hermaphrodite

during the two hour observation period. In all other cases, the male periodically clasped the carapace of a non-gravid hermaphrodite for several seconds at a time (first clasping by males; $\bar{x}=8\pm10.2$ min after isolation; range 0–35 min; $N=132$). Whenever the hermaphrodite struggled, the male released his hold and outcrossing did not occur ($N=37$). At other times the hermaphrodite did not struggle when clasped by the male, but instead, passively allowed the male to push it around the cup ($N=95$). The hermaphrodite's legs would continue to beat, suggesting active feeding, but its antennae would remain motionless, suggesting that the male was providing all active locomotion for the pair (for definitions of 'swimming' and 'feeding' see Table 1). This behavior was always a precursor to outcrossing. The average pre-outcrossing clasp time for these observations was 27 ± 36.8 min (range=2–120 min; $N=95$). Near the end of the clasping period, the male forced much of his lower body up into the hermaphrodite's carapace by the ovaries and began to make thrusting movements with the posterior portion of his abdomen ($\bar{x}=26\pm10.4$ thrusts; range=15–41 thrusts; counts made for 52 observations). In all cases ($N=95$), the eggs moved out of the ovary and up into the brood chamber while the male was thrusting, suggesting that this is when sperm transfer and fertilization occur. As soon as the eggs entered the brood chamber, the male released the hermaphrodite and outcrossing was assumed to have occurred. Male thrusting and egg movement are both visible under the microscope. In 94 of the outcrossings, hermaphrodite molting occurred prior to fertilization, (\bar{x} molting = 11 ± 5.8 min prior to fertilization; range=6–20 min; $N=94$). In the 95th outcrossing, no molting was seen during the observation but the clasping male began to thrust into the hermaphrodite's carapace anyway and egg movement still followed.

In the 20 observations in which selfing occurred, the males performed periodic clasping of the hermaphrodite's carapace for several seconds at a time but the hermaphrodites always struggled and the males never clasped the hermaphrodites for extended periods of time (>1 min). Prior to selfing, the hermaphrodite lay motionless on the substrate for up to several hours ($\bar{x}=65.3\pm34.6$ min; range=13–120+ min; $N=20$). Molting occurred on average 12.1 ± 4.9 minutes prior to selfing (range=2–22 min; $N=20$) and then the eggs moved into the brood chamber. No male ever attempted to clasp these hermaphrodites after they had molted.

Table 2. Ethogram based upon observations of laboratory populations of the clam shrimp *Eulimnadia texana*

Behavior	Description
Behaviors performed by both hermaphrodites and males:	
Feeding	Clam shrimp are filter feeders. Rapid beating of the legs creates a current pulling microscopic particles toward the clam shrimp. Clam shrimp feed almost constantly.
Lying on Bottom	Lying on the substrate at the bottom of the tank. The legs may or may not be still.
Molting	Process of shedding old exoskeleton.
Out of View	Individual clam shrimp not visible. Possibly buried in the substrate.
Struggling	Rapid movements of the body in an attempt to disengage a clasping male.
Swimming	Locomotion around tank using antennae. It is difficult to distinguish this behavior from feeding behavior because the two may occur simultaneously.
Hermaphrodite-only behaviors:	
Digging	Performed by gravid hermaphrodites only. The gravid hermaphrodite will dig in the substrate at the bottom of the tank. A hermaphrodite may dig continuously for up to several hours.
Dropping a clutch	Release of the eggs from the brood chamber. This may occur in the substrate after digging.
Male only behaviors:	
Aggressive interaction	An interaction between a male clasping a hermaphrodite and another male attempting to interfere with the pair. These interactions involve bodily contact between males, such as pushing.
Clasping	Male clasps the carapace of another clam shrimp with the first two pair of thoracic appendages. Outcrossing is always preceded by this behavior. Males also clasp other males, as well as gravid or unreceptive hermaphrodites at times.
Tagging Along	One male is clasped to a hermaphrodite and another male clasps onto him forming a 'train'.
Takeover	A male takes a hermaphrodite away from another male as a result of an aggressive interaction.
Thrusting	Male clasped to a hermaphrodite forces the lower portion of his body up into the hermaphrodite's carapace near the paired ovaries and makes pulsating motions. Sperm transfer is assumed to be taking place.

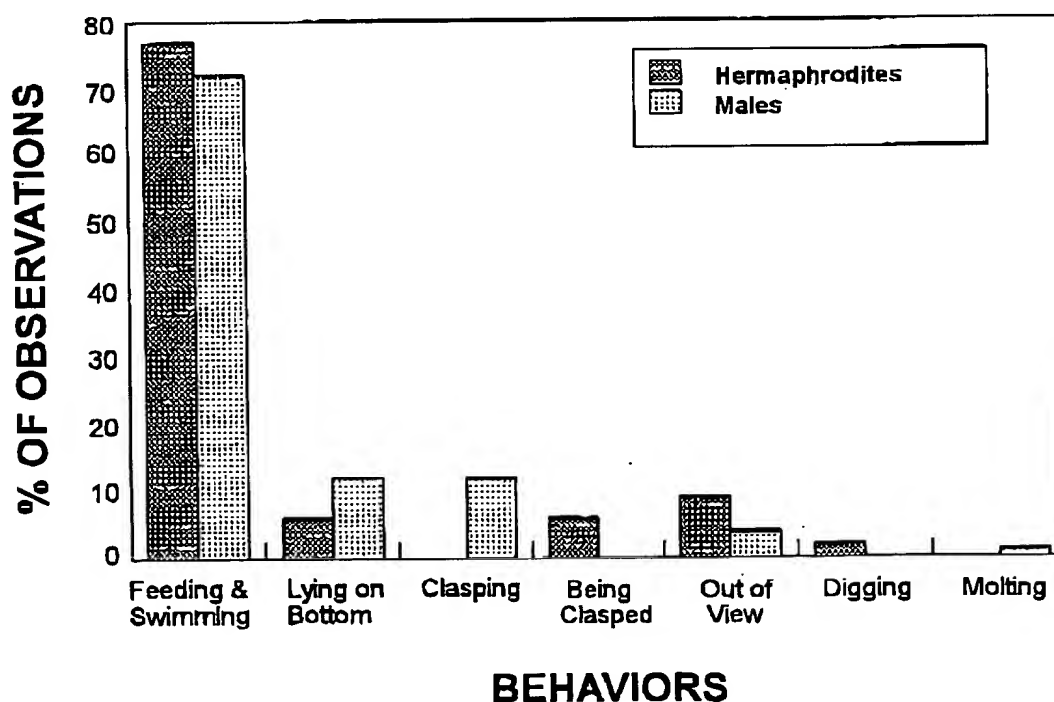


Fig. 1. Behavioral time budget for an androdioecious clam shrimp *Eulimnadia texana*. The bars indicate the average frequency of each behavior when compared to the total number of behaviors observed. Darker bars represent behaviors performed by hermaphrodites. Lighter bars represent behaviors performed by males.

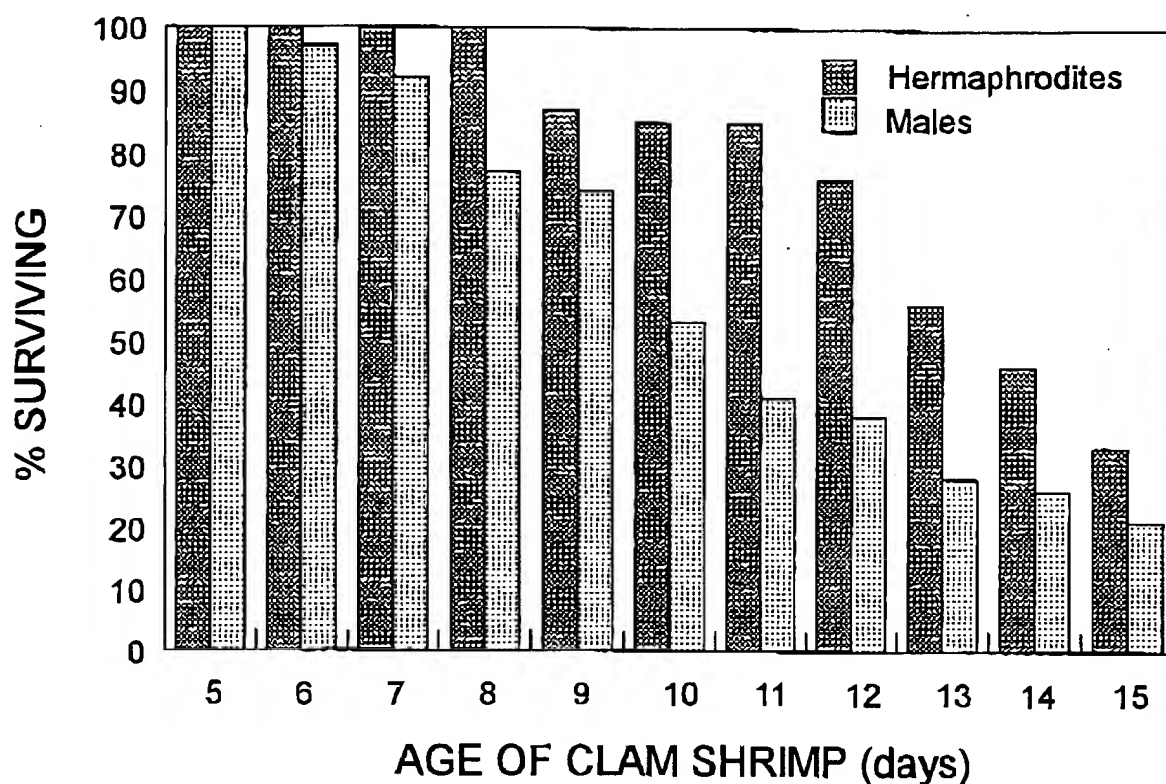


Fig. 2. Comparison of hermaphrodite and male mortality rates for laboratory populations of the clam shrimp, *Eulimnadia texana*. Populations were established when the clam shrimp were 5 days of age. $N=130$ clam shrimp from 10 experimental and 3 control tanks. See text for explanation. The dark bars represent the percentage of hermaphrodites surviving each day ($N=91$); light bars represent surviving males ($N=39$).

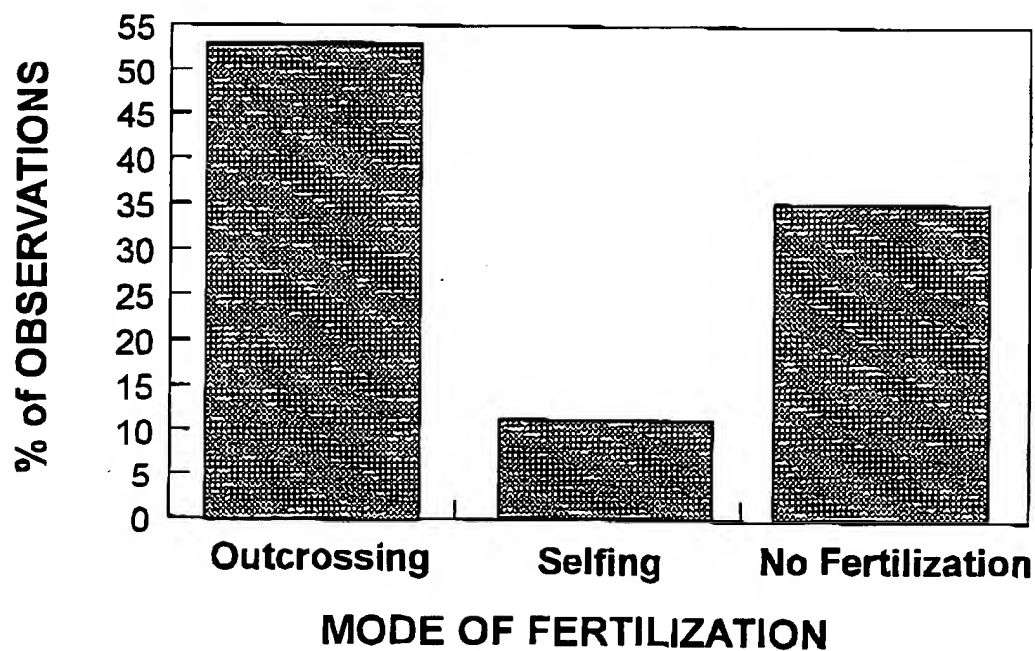


Fig. 3. Frequency of outcrossing, selfing, and lack of fertilization during two hour focal animal observations of pairs or trios of clam shrimp *Eulimnadia texana*. $N=178$ groups.

After fertilization occurred (either due to selfing or outcrossing), males were returned to rearing tanks while the hermaphrodites were kept in isolation so that eggs could be collected. Eggs remained in the brood chamber for approximately 24 hours. Hermaphrodites were returned to the rearing tanks after they dropped their eggs. On one occasion, a hermaphrodite was seen dropping its clutch and then selfing another clutch within one hour.

Discussion

Sex ratios

The majority of Conchostracan species have a 50:50 sex ratio between males and females (Sassaman, 1989). The skewed sex ratio of *E. texana* can be attributed to the nature of sex determination for this system. Comparisons of sex ratios for thirteen *E. texana* populations across the United States showed an average sex ratio of approximately 75–80% hermaphrodites, 20–25% males (Sassaman, 1989). The sex ratio in the LTER playa is fairly characteristic for the species. One population, SWP3, was entirely hermaphroditic. The percentage of males within the SWP4 and SWP5 populations (40–42% respectively) are somewhat high when compared to the range of sex ratios in populations examined by Sassaman (range=0–35% males) (Sassaman, 1989).

Population genetics modeling of the *E. texana* breeding system has suggested that the costs associated with sexual reproduction would make the gradual elimination of males a natural occurrence if some type of inbreeding depression were not present to offset the benefits of selfing (Otto *et al.*, 1993). Sassaman (1989) reported probable all-hermaphrodite populations of *E. texana* in Florida and Texas. What makes the observed absence of males in SWP3 surprising, however, is the close proximity (35 m) of this population to other male-containing populations. Eggs of *E. texana* have a diameter of 220 μ (Belk, 1989) and could be dispersed by wind action or on the feet of cattle and birds that drink from the three small neighboring populations. If hermaphrodites bury their eggs within burrows, however, as current observations suggest, these mechanisms of dispersal may be limited in effectiveness. Other factors which maintain males within some of these *E. texana* populations and seemingly inhibit gene flow between these populations are as yet unknown.

The number of viable eggs hatching from the LTER playa population were significantly less than those hatching from the three SWP populations. Data are unavailable on the length of time *E. texana* eggs can remain viable in the field, but in general the population densities of all of the species living within the LTER Playa appear to decrease with an increase in length of time between floodings (MacKay *et al.*, 1990). The LTER Playa has not seen a significant flood in ten years (October, 1984) and this may account for lower hatch rates from this soil when compared to the samples taken from the other three populations. The SWP populations typically flood at least once every year and were flooded during the summers of 1992 and 1993 (pers. obs.). Also the LTER Playa clam shrimp share the playa with other ephemeral shrimp. These species may provide competition for food resources or predation pressures which lower *E. texana* population densities. Such pressures would be absent from the SWP populations because no other ephemeral shrimp live within these sites. The bare mud substrates of the SWP sites may also be better suited for clam shrimp burrowing and egg laying than the vegetated LTER Playa substrate.

The tadpole shrimp *Triops longicaudatus* also hatched within the LTER Playa rearing tanks. Previous research had suggested that the tadpole shrimp, *T. longicaudatus* only feeds on dead prey (Medland, 1989; MacKay *et al.*, 1990; Weeks, 1990; Loring, pers. comm.). This conflicts with the behavior of *T. longicaudatus*, in our laboratory where they were avid predators; thus all tadpole shrimp were removed from rearing tanks to avoid predation. When tadpole shrimp had been left within a tank, they ate all of the clam shrimp within a matter of days. Similar predation by *T. longicaudatus* has been reported for other laboratory populations of *E. texana* (Sassaman & Weeks, 1993). This may not occur as often in the field because *T. longicaudatus* typically remain near the bottom of a playa while *E. texana* remains around the periphery (Medland, 1989; pers. obs.). Tank size constraints in the laboratory may make contact between the two species more common and thus predation more prevalent.

Behavioral observations

This study supports several predictions of a recent population genetics model designed to study the relative costs of outcrossing and selfing (Otto *et al.*, 1993). One prediction of the model is that male mortality rates will be higher than hermaphrodite mortality rates. High

male mortality rates relative to hermaphrodites within this study along with earlier field reports (Strenth, 1977) and general laboratory observations (Sassaman & Weeks, 1993) support this prediction. Secondly, Otto *et al.* (1993) predict that, unless inbreeding depression is quite high, levels of outcrossing must be high when males are present. In support of this prediction, although selfing was seen during these observations, it was comparatively rare (11%) compared to outcrossing (53% of observations). The close proximity of males and hermaphrodites in this artificial situation may, however, have contributed to the high level of outcrossing observed. The model also predicts that when males are absent, large numbers of eggs will go unfertilized. The current study found that hermaphrodites are gravid for a large percentage of their mature life span (72%) even as male numbers dwindle in the population. In order for the predictions to be correct, many of these selfed eggs must be infertile. Future studies should be designed to ascertain the relative fecundity of selfed versus outcrossed clutches.

During the instantaneous scan sampling, hermaphrodites were never seen dropping their clutches but there is some suggestion that they may deposit clutches within the burrows they dig. On several occasions during the initial observations, gravid hermaphrodites were seen digging a burrow and then emerging from it without their clutch. Non-gravid hermaphrodites and males were never seen digging burrows. During the focal animal observations, no substrate was provided so that all interactions between the clam shrimp would be visible and eggs could be collected. Absence of a substrate precluded observations of any digging behavior. During the scan sample observations, males were seen entering burrows. If hermaphrodites drop their clutch within a burrow, it seems likely that males might enter burrows in search of non-gravid hermaphrodites to mate guard prior to the hermaphrodites' next clutch.

Outcrossing behavior

This study provides the first detailed documentation of the sequence of events associated with outcrossing for *E. texana*. Molting appears to be necessary before fertilization is possible. This is a common occurrence in crustaceans (Ward, 1983). In many crustaceans, the correlation between molting and fertilization has limited the receptivity period of females and it has been suggested that this may lead to male/male competition for mates and mate guarding (Elwood *et al.*, 1987; Anstensrud, 1992). The male's clasping and swim-

ming with the hermaphrodite for extended periods suggests that precopulatory mate guarding exists within this species. Male clasping of gravid hermaphrodites provides further evidence for the occurrence of precopulatory mate guarding. In studies of other small crustacean species, mate guarding carries a variety of costs including high energy requirements, an increase in susceptibility to predation, and loss of time that could be spent searching for other females (Elwood & Dick, 1990). There may be similar costs related to mate guarding in *E. texana*, particularly relating to increases in energy demands since males push hermaphrodites around and fight off other males (see Knoll & Zucker, 1994).

The *E. texana* mating system provides an ideal opportunity to study the relative costs of sex and selfing. It is well known that outcrossing incurs the costs of sex. The maintenance of males in most populations of this species suggests that there must be costs to selfing as well. My observations suggest that the hermaphrodite can control the mode of fertilization (selfing vs outcrossing). Outcrossing was never observed between a male and a struggling hermaphrodite. There were instances in which the struggling hermaphrodite was much smaller than the clasping male and could not have physically forced him to release his grasp (pers. obs.). Instead, it appeared that males released the hermaphrodite upon its struggling. My results also suggest that there are time costs incurred by hermaphrodites that self. Once a hermaphrodite let a male clasp for more than one minute, it was committed to outcrossing which occurred an average of 27 minutes later. A hermaphrodite that selfed, however, remained motionless an average of 65 minutes prior to fertilization. Thus outcrossing occurred in less than half the time on average than selfing.

During careful examination of mating behavior, I failed to observe any indication of a spermatophore-type structure being transferred to the hermaphrodite. A previous study suggested that males transfer such a spermatophore-like package to the eleventh thoracic segment of the hermaphrodite during outcrossing (Strenth, 1977). If sperm were transferred in a spermatophore-like form, it may have been too small to be visible during these observations.

The unique mating system of *Eulimnadia texana* and its close relatives provides the potential for answering many intriguing questions on the evolution of sex. Toward that end, this contribution provides the first detailed description of the behavior of these organisms

with an emphasis on mating activities. Many questions about the reproductive behavior and physiology of this species remain to be answered.

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Is sexual selection operating in the androdioecious clam shrimp, *Eulimnadia texana* (Crustacea: Conchostraca)?

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Key words: androdioecious crustacean, *Eulimnadia texana*, sexual selection, mate choice, male–male competition

Abstract

Experiments were performed to document the existence of intersexual or intrasexual selection in the clam shrimp, *Eulimnadia texana*. Individuals within this species are either males or hermaphrodites. Hermaphrodites can self their own eggs or outcross with a male, but they cannot outcross with other hermaphrodites. Theoretical considerations suggest that both intrasexual and intersexual selection could be occurring on the part of the hermaphrodites and the males. When males were given a choice between two non-gravid hermaphrodites of different sizes, they did not exhibit a mating preference based upon size. When two males of different sizes were isolated with a single nongravid receptive hermaphrodite, the hermaphrodite showed no preference between the two males. There was evidence, however, of male–male competition for receptive hermaphrodites and of mate guarding on the part of the males. During aggressive encounters between two males, the larger of the two had a significant advantage over the smaller, and larger males were always the victors when hermaphrodite takeovers occurred as a result of male–male conflict. Hermaphrodites appear to control the mating process both by struggling with males when they are not receptive to them and by selfing in the presence of males. This suggests that hermaphrodites withhold receptivity cues from males, or produce non-receptivity cues, when they are going to self. Though hermaphrodites do not appear to select males based upon size, they may make a selection between selfing and outcrossing by controlling the use of receptivity signals.

Introduction

Individual clam shrimp *Eulimnadia texana* (Crustacea: Conchostraca) are either male or hermaphroditic. This type of mating system is known as androdioecy, a rare condition in animals (Sassaman & Weeks, 1993). Hermaphrodites are capable of selfing their own eggs or outcrossing with a male, but they cannot exchange sperm with other hermaphrodites. Based upon what we know about the reproductive biology of this species, there is the potential that sexual selection is an important evolutionary force within this mating system. A case could be made for hermaphrodite–hermaphrodite competition (intrasexual selection) and male mate choice (intersexual selection), as well as for male–male competition (intrasexual selection) and hermaphrodite mate choice (intersexual selection).

The case for hermaphrodite–hermaphrodite competition: The sex ratio within this species is highly

skewed in favor of the hermaphrodites (Sassaman, 1989; Knoll, 1994). This often results in competition among members of the common sex for members of the rare sex (Emlen & Oring, 1977). Thus, one might predict hermaphrodite–hermaphrodite ('female–female') competition for males.

The case for male mate choice: Behavioral observations suggest that males practice precopulatory mate guarding of hermaphrodites within this species (Knoll, 1994). Mate choice by the males often occurs in systems where mate guarding is present (Parker, 1974; Thompson & Manning, 1981; Elwood, *et al.*, 1987). Sexual selection theory predicts that the sex putting the larger investment into reproduction will be the selective sex during mating (Trivers, 1972). This is typically the female. When male investment in reproduction is high, such as in systems where the male aides in offspring rearing or expends large amounts of energy in spermatophore production, males have been known to

become selective for females (Ridley, 1978; Dewsbury, 1982). The energy investment of mate guarding, as seen in *E. texana*, and the risks likely to be incurred by this behavior may lead to selectivity on the part of the male (Parker, 1974; Hatzios & Caldwell, 1983). Hermaphroditic clam shrimp show a significant increase in fecundity as they increase in size. Males that choose larger hermaphrodites could significantly increase their reproductive success. Thus one might predict male mate choice of large hermaphrodites. Should hermaphrodite-hermaphrodite competition and male mate choice both be occurring, clam shrimp would exhibit sex role reversal for both intra- and intersexual selection. This is extremely rare in crustaceans (Hatzios & Caldwell, 1983).

The case for male-male competition: There is a complication in this system, however, because hermaphrodites will self quite readily when males are not present and even when males are present (Sassaman & Weeks, 1993; Knoll, 1994). The hermaphrodites remain gravid for much of their life cycle and can self within an hour after the previous clutch of eggs have been laid (Knoll, 1994). The fact that hermaphrodites self and only have short periods when they are apparently receptive to mating may actually change the operational sex ratio, or the ratio of fertilizable hermaphrodites to sexually active males (Emlen & Oring, 1977), in favor of males. This could lead to competition among males for the 'rare' non-gravid, receptive hermaphrodites. Fertilization appears to occur shortly after molting (Knoll, 1994). Male-male competition is particularly common in crustaceans largely because females are only receptive for short periods of time when molting occurs (Ward, 1983; Elwood, *et al.*, 1987; Anstensrud, 1992). In many of these systems, mate guarding has emerged as a male competition strategy rather than part of a male selective strategy (Parker, 1974).

The case for hermaphrodite mate choice: There is also the possibility that hermaphrodites exhibit mate choice for large males. Since clam shrimp continue to grow with age, the larger males are older and have thus demonstrated the ability to survive. Furthermore, clasping males provide locomotion for the hermaphrodite (Knoll, 1994); larger males may be better equipped to provide this energy saving service to the hermaphrodites.

This study was designed to explore each of these possibilities in *E. texana*.

Methods

Rearing procedure

Numerous samples of the first 1–2 cm of soil were collected from the playa portion of the NSF Long-Term Ecological Research site on the New Mexico State University College Ranch located at the northern edge of the Doña Ana Mountains 40 km NNE of Las Cruces, Doña Ana County, New Mexico. For a description of the site see MacKay, *et al.*, 1990. Sample populations were established in the laboratory by placing 250 ml soil samples in plastic mouse cages (30 cm × 12 cm × 15 cm) and hydrating them with 4 liters of aged tap water. The tanks were kept on a 24 hour light cycle using incandescent bulbs in refectories to maintain summer-like temperatures of approximately 30 °C and were supplemented with tadpole food pellets until algal growth was initiated. The clam shrimp reached sexual maturity within approximately three to five days after hatching at about 3 mm in carapace length. In sexually mature hermaphrodites, the eggs are visible in paired ovaries located along the digestive tract and fertilized clutches are carried by the hermaphrodite in a dorsal brood chamber for approximately 24 hours prior to being released onto the substrate (*pers. obs.*). There are no visible external cues to sexual maturity in the males, but males with carapace lengths as small as 3 mm were seen clasping hermaphrodites (*pers. obs.*). Clam shrimp smaller than 3 mm were not used in observations and individual clam shrimp were only used once.

A test for hermaphrodite intersexual selection and male intrasexual selection

Two sexually mature males with carapace diameters of at least a 0.5 mm difference were placed with a sexually mature non-gravid hermaphrodite of a random size. The trio was measured and then isolated in a plastic 'dixie' cup containing 125 ml of aged tap water. Size and sexual dimorphism were pronounced enough that no marking was necessary for individual identification. The behavior of the trio was observed and detailed records were kept of all interactions between the clam shrimp using the focal animal techniques outlined by Altmann (1974). Observations generally lasted for two hours or until fertilization was observed, whichever came first. At the end of 2 hours, however, if a hermaphrodite and male were engaged in an interaction, the observation was extended until fertil-

ization occurred or the interaction ended. Sperm transfer and fertilization were assumed to have occurred when the clasping male began to thrust his body up into the hermaphrodite's carapace and the eggs moved from the ovary up into the brood chamber. Selfing was assumed to have occurred when the hermaphrodite remained motionless on the bottom of the cup and the eggs moved into the brood chamber with no intervention from either of the males. For detailed descriptions of both outcrossing and selfing see Knoll (1994).

A test for male intersexual selection and hermaphrodite intrasexual selection

Two sexually mature non-gravid hermaphrodites with carapace diameters of at least a 0.5 mm difference were placed with a sexually mature male of a random size. The trio was measured, isolated and observed in the same manner outlined above. Once again, observations lasted for two hours or until fertilization occurred in one of the two hermaphrodites. The methods for determining outcrossing and selfing were also the same.

Analysis

For statistical procedures, an α level <0.05 is considered significant throughout this study.

Results

Hermaphrodite intersexual selection and male intrasexual selection

In the study population, hermaphrodite carapace length ranged from 3.0 mm to 7.0 mm and males from 3.0 mm to 6.5 mm. The average size difference between the pairs of males in this study was 1.45 mm. In 29 observations (58%), no fertilization occurred and it is assumed that the hermaphrodite was not receptive for the duration of the observation period. The results of the other 21 trials are presented in Fig. 1. The larger male outcrossed with the hermaphrodite significantly more often (52%) than did the smaller male (14%) ($\chi^2 = 5.99$, $df = 2$, $P = 0.05$). In the observations where outcrossing occurred, aggressive interactions were evident between the males in 79% of the trials (for a definition of 'aggressive interactions', see Knoll, 1994). These types of encounters only occurred when one male was clasping the hermaphrodite and the other approached the pair. In the instances where

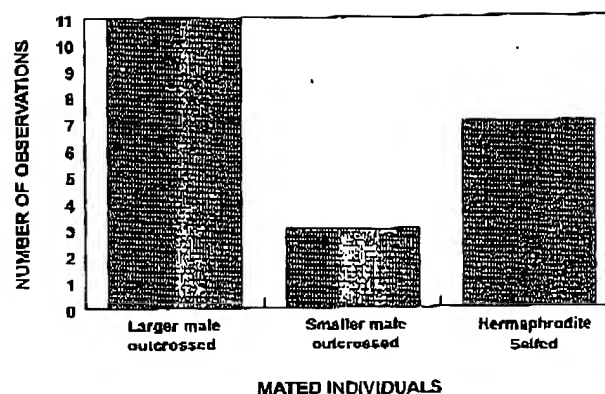


Fig. 1. Mating outcome of two male-one hermaphrodite groups of the androdioecious clam shrimp *Eulimnadia texana*. Bars represent the frequency of outcrossing involving each male and selfing by hermaphrodites ($N = 21$).

the hermaphrodite selfed its eggs (33%), neither male ever attempted prolonged clasping (>1 min) of the hermaphrodite, a requisite for outcrossing (Knoll, 1994). Aggressive interactions between males were not seen in the trios where the hermaphrodite selfed. There were three instances in which one male guarded the other male and attempted to outcross with him rather than with the hermaphrodite. In all three cases, the larger male did the guarding and the smaller male initially struggled but was unsuccessful in dislodging him.

Four successful takeovers were seen during the course of the observations and in each instance it was the larger male that removed the hermaphrodite from the smaller male. A takeover is defined as an incident in which a male dislodges a clasping male from a hermaphrodite and begins to clasp it himself.

Hermaphrodites struggled with one or both of the males in 15 of the 29 observations (52%) in which no fertilization took place (for a definition of 'struggle', see Knoll, 1994). In the other 14 observations, there were no interactions between the males and the hermaphrodite. Hermaphrodites were never seen struggling with one of the males and then outcrossing with the other. That is, there was no evidence of any type of selection or assessment behavior by the hermaphrodite for the two males.

Male intersexual selection and hermaphrodite

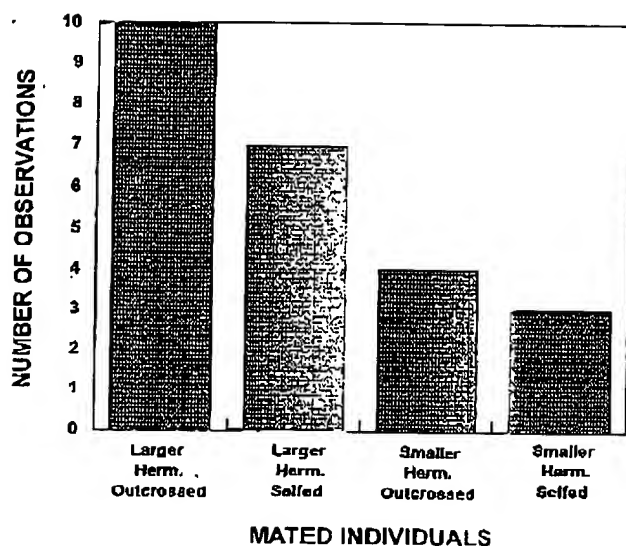


Fig. 2. Mating outcome of two hermaphrodite-one male groups of the androdioecious clam shrimp *Eulimnadia texana*. Bars represent the frequency of outcrossing and selfing involving each hermaphrodite (N=24).

intrasexual selection

The average size difference between paired hermaphrodites in this study was 1.38 mm. In 12 observations (33%), no fertilization occurred and it is assumed that the hermaphrodites were not receptive during the two hour observation period. The results of the other 24 observations are presented in Fig. 2. There was no significant difference in the outcomes of these 24 observations ($\chi^2 = 5.01$; $df = 3$, $P > 0.1$). The larger hermaphrodite selfed more often (29%) than the smaller hermaphrodite (13%), but this was not significant ($\chi^2 = 1.6$; $df = 1$, $P > 0.2$). The larger hermaphrodite also outcrossed more often (42%) than the smaller hermaphrodite (17%), but this too was not significant ($\chi^2 = 1.86$; $df = 1$, $P > 0.1$). No male was ever seen clasping each hermaphrodite in turn and then selecting one or the other for outcrossing.

There were ten observations (42%) in which one of the two hermaphrodites selfed and the male did not attempt to outcross with either hermaphrodite. There was one instance when the male was clasped to the smaller hermaphrodite and the larger hermaphrodite continually swam near the pair, bumping into them in what appeared to be an attempt to interfere with outcrossing. The male released the smaller hermaphrodite after clasping it for 32 minutes and no outcrossing occurred with either hermaphrodite.

Larger hermaphrodites had their eggs fertilized, either through selfing or outcrossing, in 71% of the observations (Fig. 2, sum of left two bars). This was significantly higher than the frequency of egg fertilization for smaller hermaphrodites (29%) ($\chi^2 = 4.17$, $df = 1$, $P < 0.05$) (Fig. 2, sum of right two bars).

Discussion

Large body size in *E. texana* appears to convey a significant advantage to males. Other studies have shown that there is a positive correlation between body size and reproductive success in a wide variety of taxa (see Alexander *et al.*, 1979; Trivers, 1985 for a review). In *E. texana*, the advantage of larger body size is attributable to success in male-male competition (intrasexual selection) rather than a preference by hermaphrodites for larger males (intersexual selection). There was no detectable difference in hermaphrodite behavior toward larger and smaller males. Aggressive male interactions in *E. texana* only occurred when one male was clasping the hermaphrodite and the other was attempting to interfere. Since hermaphrodites are only receptive during limited periods of time and may choose to self their eggs (Knoll, 1994), any advantage a larger male has in dislodging other males may translate into a significant reproductive advantage for him. Observations indicate that precopulatory mate guarding occurs in *E. texana* (Knoll, 1994). If there are costs associated with mate guarding, larger males may be better equipped to meet these demands.

Though males practice mate guarding and may encounter high reproductive costs during the process (Knoll, 1994), it has not led to a male preference for larger hermaphrodites. These data are in conflict with the findings of similar studies using the amphipod *Gammarus pulex* (Elwood, *et al.*, 1987), the isopod *Asellus aquaticus* (Thompson & Manning, 1981) and the stomatopod *Pseudosquilla ciliata* (Hatzisios & Caldwell, 1983). Unlike the stable habitat of these crustaceans, the ephemeral pond habitat of clam shrimp provides a highly unpredictable habitat with drying possible at any time. Searching for and assessment of hermaphrodites may be too costly time-wise in such an unpredictable environment, particularly since hermaphrodites are only receptive for limited amounts of time (Knoll, 1994). Though mating with larger hermaphrodites would be advantageous to a male because he can father significantly more offspring

(Knoll & Zucker, 1994), the unpredictability of this environment may outweigh any advantages that a male could obtain from active mate preference.

The selfing ability of the hermaphrodites complicates this system. The occurrence of selfing during this experiment demonstrates that hermaphrodites will self readily despite the presence of a male. Thus non-gravid, receptive hermaphrodites may be more rare than was initially predicted. The reproductive success of a male is largely controlled by the number of times he is able to mate (Trivers, 1972). Males may be forced to mate with any available hermaphrodite in order to achieve any reproductive success. Field data on the actual number of receptive hermaphrodites in the natural environment would be very helpful. These studies, however, are difficult due to the unpredictability of pond filling. The last time the LTER Playa filled sufficiently to allow Branchiopod activity was in 1984 and this playa typically only fills once every three to ten years (MacKay, *et al.*, 1990).

The overall level of selfing in the presence of males (17 occurrences—18% of the observations) was higher than expected. In many simultaneously hermaphroditic animals, outcrossing is the primary reproductive method while selfing is typically used when animals are isolated and stored sperm has been used up or become too old (Jarne, *et al.*, 1991; Jarne, *et al.*, 1992; Warner, 1993). Many of these species live in permanent aquatic environments or in environments with stable water resources. The unpredictability of *E. texana*'s water resources may be partially responsible for the high frequency of selfing in this species. Waiting for the opportunity to outcross with available males could be counterproductive when ponds may dry rapidly.

When selfing occurred, males never approached the hermaphrodite. This suggests that males were not aware that the hermaphrodite was receptive. Information on the types of cues utilized by hermaphrodites to signal receptivity to the male are unavailable. The use of pheromones to signal receptivity has been reported in crustaceans (Hazlett, 1970; Tierney & Dunham, 1982). Receptivity cues in *E. texana* are probably chemical in nature, as well. A chemical cue could be either a receptivity or a non-receptivity cue. In this study, some males clasped other males for prolonged periods of time, but males never clasped hermaphrodites for more than a few seconds unless outcrossing followed, suggesting that the hermaphrodites release a non-receptivity cue. In its absence, clasping continues. Since males are generally more rare than hermaphrodites (Knoll, 1994), the chance of 1 male

randomly clasping onto another would be low. When a hermaphrodite is going to self, it may not release this chemical signal or it may release a chemical signaling its lack of receptivity. Release or non-release of a non-receptivity signal may be a choice on the part of the hermaphrodite between selfing and outcrossing respectively. If such a 'choice' is made, it can be considered an unusual form of mate choice.

Neither males nor hermaphrodites were ever seen performing any type of assessment behavior between two individuals that would suggest the occurrence of intersexual selection within this species. This study was designed to test for assessment of morphological characteristics such as size. If mate assessment occurs at a distance using chemical cues, it would not have been evident in this study. Further studies designed to evaluate the role of chemical cues in mate choice are underway.

As indicated above, a male would clasp another male occasionally for extended periods of time and perform what appeared to be mate guarding behavior. There seems to be little utility in one male attempting to outcross with another male. Gregarious behavior has also been reported for the copepod *Lernaeocera branchialis* (Anstensrud, 1992). Anstensrud suggested that chemicals given off by juvenile males in *L. branchialis* may be confused with those given off by females in various stages of development. In *Gammarus pulex*, males were reported to have continued precopulatory mate guarding with deceased females (Ward, 1983). Little adaptive significance can be attributed to these types of behaviors and they suggest that mating cues are not 100% accurate. The frequency of these non-adaptive behaviors is low and it may be that the cost of more effective signaling systems would override any benefits obtained.

In summary, male-male competition appears to be the only form of sexual selection operating in this population of the clam shrimp, *Eulimnadia texana*. Nevertheless, the observation of one hermaphrodite appearing to interfere with the outcrossing attempt of a male and smaller hermaphrodite suggests that further studies are needed to fully understand this mating system. Studies under field conditions or larger laboratory populations which better simulate the natural environment should be performed to that end.

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Anatomical evidence for androdioecy in the clam shrimp *Eulimnadia texana*

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Key words: androdioecious crustacean, *Eulimnadia texana*, hermaphrodite, ovotestis, reproductive system, testis

Abstract

Recent genetic evidence suggests that the clam shrimp *Eulimnadia texana* exhibits androdioecy, a rare mating system. In this system, individuals are either hermaphrodites or males, pure females not being found. Through the use of light microscopy, this study provides anatomical evidence that egg-bearing individuals are, indeed, hermaphrodites. All such individuals observed possess ovotestes with testicular tissue restricted to the posterior region of the gonad. Developing sperms in hermaphrodites are similar in appearance to those seen in male-only individuals.

Introduction

The wide variety of mating systems exhibited by both animals and plants has intrigued evolutionary biologists for decades, for the adaptive value of biparental reproduction (the predominant form) remains unclear to some in light of the two-fold genetic advantage gained through uniparental reproduction (Stearns, 1990). As an increased understanding of the biology of plants and animals reveals ever more variations on the theme, the intrigue and the investigation of unusual mating systems grow.

Ephemeral branchiopods represent one group attracting much recent interest. Not only is there growing evidence of rare mating systems among these crustaceans, but many species within this group have become endangered or threatened as loss of their fragile habitat escalates. The clam shrimp *Eulimnadia texana* is an ideal candidate for study since it is still very abundant in depressions, cattle tanks and playas throughout the southwestern U.S. (Sassaman, 1989). It is a small (carapace length to 8 mm, but more typically about 5 mm) species of clam shrimp (family Limnadiidae; order Conchostraca) that is readily reared in the laboratory. Furthermore, recent studies suggest it exhibits a rare mating system – androdioecy, typified by populations composed of males and hermaphrodites.

A large pedigree study by Sassaman & Weeks (1993) found that while some isolated 'females' produced only 'female' offspring as would be characteristic of a parthenogenetic system, others produced about 25% male offspring. Males are found in most populations but always in lower proportions than 'females' (Sassaman, 1995). Offspring reared from 'females' mated to males were either all 'female' or about 50% male. The most parsimonious explanation for these findings, according to Sassaman & Weeks (1993), is not a parthenogenetic system, but an androdioecious one. For *E. texana*, Sassaman & Weeks (1993) proposed that 'females' are instead hermaphrodites which could either self or outcross with males. Sex was proposed to be determined by a simple Mendelian locus with two alleles, where a dominant allele codes for hermaphrodites and recessive alleles for males. Thus, a homozygous dominant hermaphrodite (a 'monogenic') would produce only hermaphroditic offspring whether selfing or outcrossing, while a heterozygous hermaphrodite (an 'amphigenic') would produce 25% sons from selfing and 50% sons from outcrossing.

Here we present corroborative evidence for androdioecy in *E. texana* by providing the first anatomical evidence for the presence of ovotestes in hermaphroditic individuals of this species, once thought to be females.

Methods

Samples were obtained from dormant cysts in soil collected from a depression west of South Well (N 32° 31.933'; W 106° 44.933') on the USDA Jornada Experimental Range, 25 km north of Las Cruces, Doña Ana County, New Mexico. Soil was hydrated in the laboratory at about 28 °C under an incandescent lamp. Nauplii larvae, which hatched less than 20 hours later, were placed singly in 30 ml cups when approximately 30 hours of age. Larvae were reared on a diet of yeast solution and fish fry food (Tetra-Min Baby 'E' food). Early larval isolation ensured that sectioned individuals could not have mated and stored sperm prior to fixation.

Sexually mature males (at least 5 days old) were readily identified by two pairs of claspers on their anterior thoracic limbs. All individuals that lacked claspers carried eggs in their gonads (readily seen through the transparent shell under a dissecting scope or hand lens). Most of these individuals also had shelled eggs in their brood chamber.

Sexually mature individuals were placed in fixative containing 4% paraformaldehyde and 2.5% glutaraldehyde in Sorensen's phosphate buffer pH 7.4 with the addition of 0.5% dimethylsulfoxide to aid in the penetration of the fixative. After at least one hour in the fixative, the carapace of most individuals was dissected (to facilitate sectioning) away from the body, which was returned to the fixative for an additional 3–6 hours. Specimens were washed in buffer for 1 hour and transferred to fresh buffer overnight. All samples were washed twice in fresh buffer for at least 1 hour per wash.

Samples were dehydrated through a graded ethanol series (35%, 50%, 70%, 95%) for about 2 hours in each solution. This was followed with 3 changes in absolute ethanol for at least 2 hours per change. The samples were infiltrated with a 50:50 mix of Spurr's resin and ethanol for 4 hours. The resin:ethanol was replaced with 100% Spurr's overnight. The resin was replaced with fresh resin and held under vacuum for about 6 hours. The specimens were placed in aluminum dishes with fresh resin and placed in a 70 ° oven for 24 hours.

Using glass knives, 1–2 μ m longitudinal sections were cut in the sagittal plane and stained with 0.1% toluidine blue in 1% borax for 30 s on an 80 °C hot plate, rinsed with distilled water and covered with a coverslip using permount.

Results

Ten egg-laden individuals in various stages of egg-development and three male clam shrimp were serially sectioned and examined for the presence of testicular tissue. The reproductive system in *E. texana* consists of an elongate, lobed gonad lying on each side of the digestive tract (Figure 1). Light microscope sections of the male gonads reveal a gonadal wall of short round cells which stain relatively lightly in toluidine blue. Developing spermatozoa move into the lumen of the testis from the germ cells of the wall. Mature sperm are seen in the lumen (very lightly staining) and are amoeboid in shape (Figures 1E, 2A). The gonads of all egg-laden individuals examined exhibited ovarian tissue in the anterior 4/5 or so of the organ, with tissue resembling that of male gonad in the posterior-most region (Figures 1D, F, 2B, 3A, B). The ovarian portion of the ovotestis consists of darkly-staining columnar cells lining the wall of the gonad (Figures 2B, 3A, B). The lumen of the ovarian portion of the gonad is filled either with egg-shell substance (Figure 3A) or with mature eggs surrounded by egg-shell substance (Figure 3B), depending upon egg stage. In Figure 3B, note that one egg is in the process of being oviposited through the gonopore located between the 10th and 11th thoracic limbs. Several sections of this specimen revealed mature sperm in the anterior region of the testicular portion of the gonad in close proximity to an egg surrounded by egg-shell substance (Figure 3B). In specimens devoid of eggs within the lumen, oocytes are seen jutting into the hemocoel of the shrimp (Figure 3A) from side branches of the ovotestis. The posterior portion of the ovotestis exhibits the same lightly-staining round cells as seen in the male testis, with various stages of developing sperm moving into the lumen (Figures 1F, 2B, 3A, B). A close-up of the testis of a male (Figure 2A) and the posterior (testicular) region of a hermaphrodite (Figure 2B) reveals similar tissue in each, although many more sperm pack the male testis. Since the hermaphrodites were isolated while still larvae, these sperm could not be the result of storage after an outcrossing.

Discussion

The location and general structure of the reproductive system and the presence of non-flagellated, amoeboid sperm in *Eulimnadia texana* are typical of other branchiopods so far studied (Wingstrand, 1978; Martin,

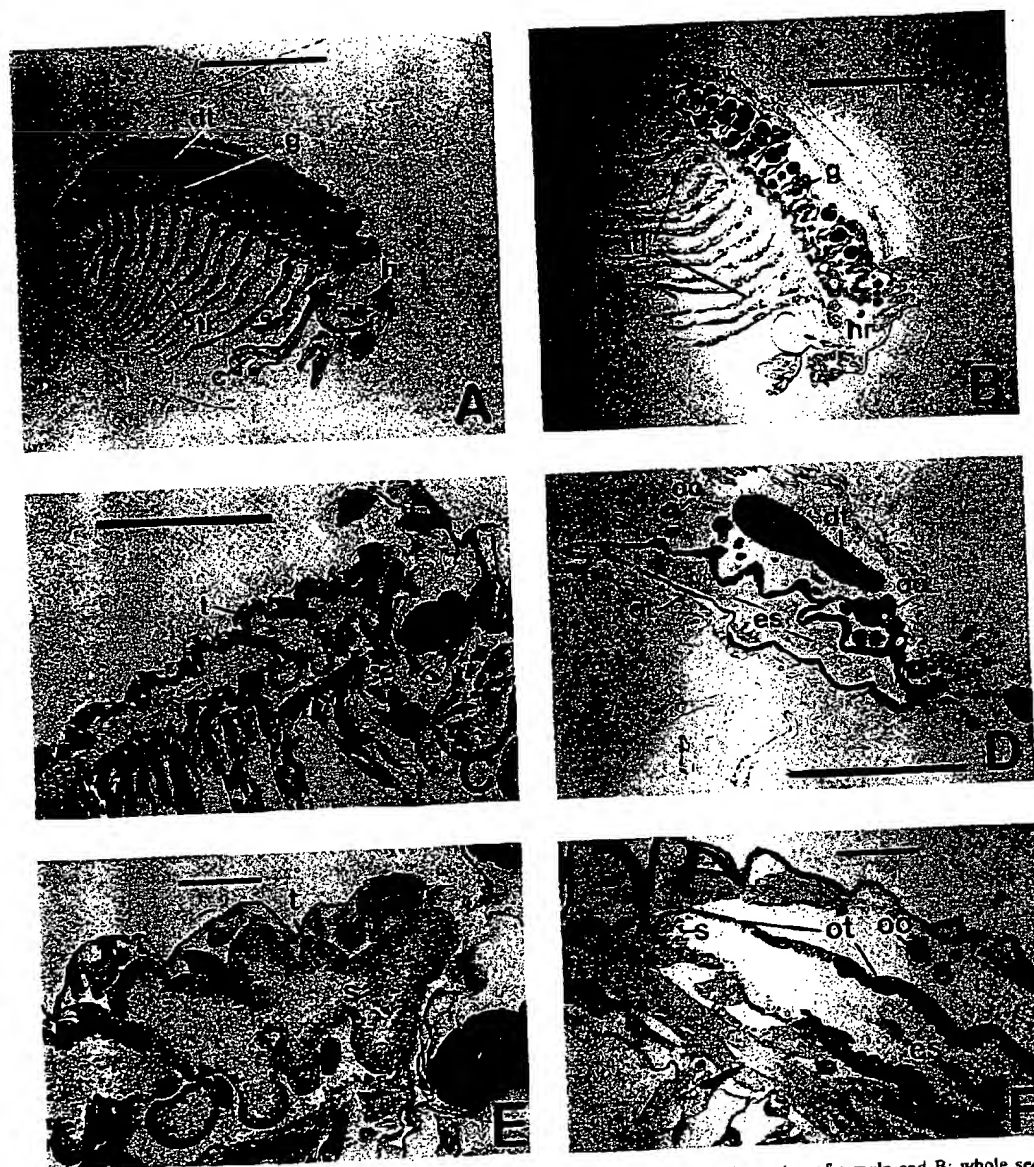


Figure 1. Sagittal sections through gonads of the clam shrimp, *Eulimnadia texana*. A: whole section of a male and B: whole section of a hermaphrodite (for orientation purposes). Scale bars in A and B, 1 mm. C: anterior portion of testis of a male (scale bar, 500 μ m). D: posterior portion of ovotestis of a hermaphrodite. Scale bar, 500 μ m. E: higher magnification of anterior portion of testis from male in C. Scale bar, 100 μ m. F: higher magnification of testicular region from another hermaphrodite. Scale bar, 100 μ m. c, clasper; dt, digestive tract; cs, egg-shell substance; g, gonad; hr, head region; oo, oocyte; ot, ovotestis; s, sperm; t, testis; tl, thoracic limbs.

1992; Tommasini & Scanabissi Sabelli, 1992). The existence of both well-developed ovarian and testicular tissue in the gonads of the same individual confirms the genetic evidence of Sassaman & Weeks (1993) that individuals once thought to be parthenogenetically-reproducing females are, instead, hermaphrodites. The

presence of several stages of sperm development in all egg-bearing individuals examined, as well as the simultaneous presence of mature sperm and eggs in the same individual, strongly supports the idea that pure females are not present, and that isolated individ-

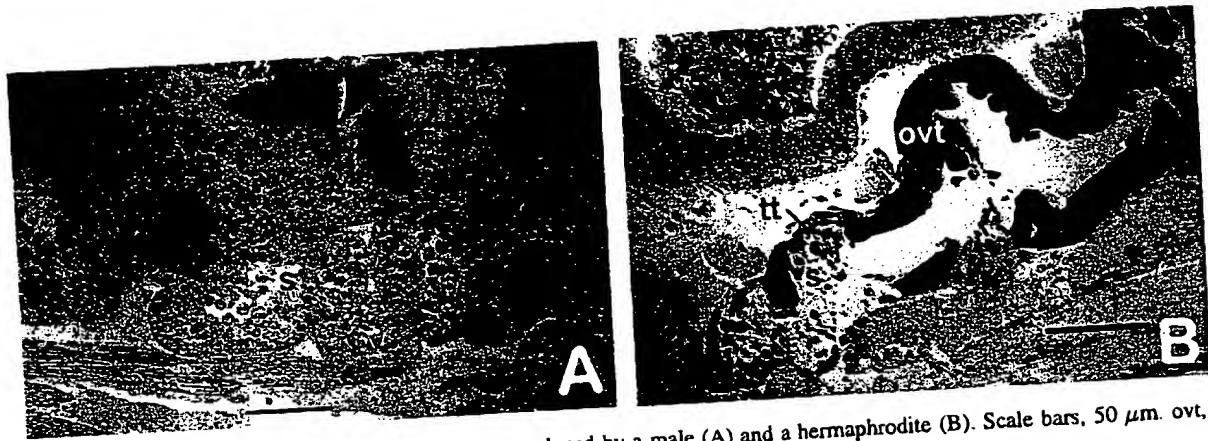


Figure 2. Close-up view showing a comparison of sperm produced by a male (A) and a hermaphrodite (B). Scale bars, 50 μ m. ovt, ovarian tissue of ovotestis; s, sperm; tt, testicular tissue of ovotestis.

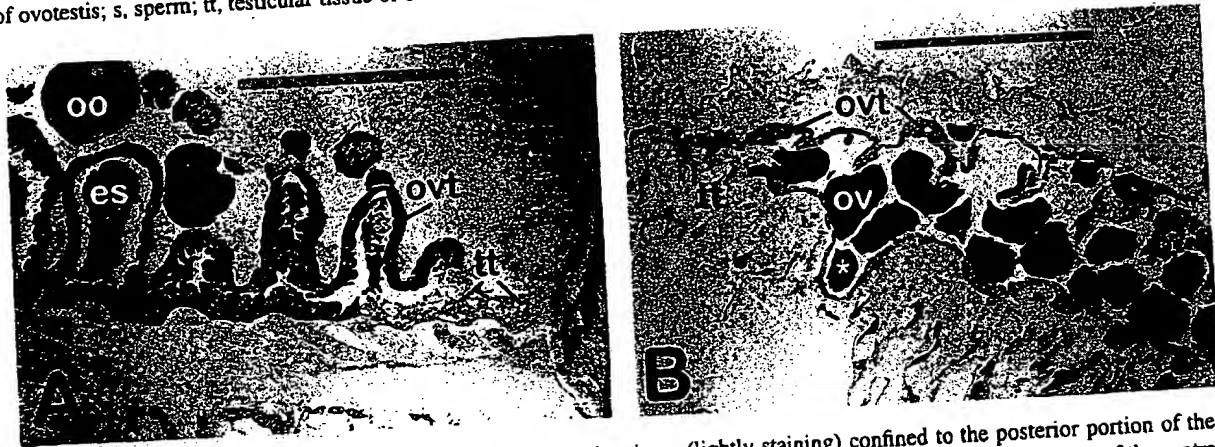


Figure 3. Ovotestis from two other hermaphrodites showing testicular tissue (lightly staining) confined to the posterior portion of the gonad. Scale bars, 500 μ m. In (A), oocytes are seen in side branches jutting into the hemocoel; in (B), ova are seen within the lumen of the central cavity of the ovotestis, coated with egg-shell substance. Note ovum (asterisk) exiting through the gonopore between the 10th and 11th thoracic segments (also see Figure 1B). es, egg-shell substance; oo, oocyte; ov, ovum surrounded with egg-shell substance; ovt, ovarian tissue of ovotestis; tt, testicular tissue of ovotestis.

uals that can produce viable offspring do so by selfing hermaphroditism.

It has long been known that some species of uniparental notostracan branchiopods are hermaphroditic, as first suspected by Bernard in the late 1800s (Wingstrand, 1978) and later confirmed by Longhurst (1955) (see Sassaman, 1991, for a review). Uniparental conchostracan branchiopods, however, were believed to reproduce by parthenogenesis (Wingstrand, 1978) despite the finding by Zaffagnini (1969) of rudimentary hermaphroditism in *Limnadia lenticularis*. In both the hermaphroditic notostracans (Longhurst, 1955) and the conchostracan *Limnadia lenticularis* (Zaffagnini, 1969), the testicular tissue appears scattered throughout the length of the ovotestis in small pockets. Zaffagnini (1969) observed few if any mature sperm in the unisexual species *L. lenticularis*, and, based on cytological studies, believed that reproduction is via automictic parthenogenesis. In contrast, in the present

study, all *E. texana* hermaphrodites so far examined possessed a well-developed, concentrated region of testicular tissue limited to the posterior portion of the gonad, beyond the 11th thoracic segment where the gonopore is located. No signs of testicular tissue were detected in other parts of the gonad despite examination of serial sections throughout the entire gonad of several individuals. And, unlike Zaffagnini's (1969) observations, well-developed mature sperm were present in the lumen of hermaphroditic *E. texana* gonads.

Most species of branchiopods where ovotestes have been found are believed to be unisexual, requiring some form of uniparental reproduction. In the case of *E. texana* (and at least one other *Eulimnadia* species and one *Triops* species) males are also present in the population along with hermaphrodites, albeit in lower numbers (Sassaman, 1991). This androdioecious mating system is quite rare in both the plant and animal kingdoms (Charlesworth, 1984). In animals it is

known in the well-studied nematode *Caenorhabditis elegans* (Barker, 1992), and in the barnacle *Balanus galeatus* (Gomez, 1975). Androdioecy, however, may be far more pervasive among the branchiopods (and perhaps other invertebrates) than is currently recognized. For example, the current study, in conjunction with that of Sassaman & Weeks (1993), provides strong evidence for this type of mating system in *E. texana*, and Sassaman has data suggesting similar reproductive systems in *E. antlei* and *Triops newberryi* (Sassaman, 1991). While still assumed to reproduce parthenogenetically, the recent discovery of two males in *Limnadia lenticularis* (Sassaman, 1995), along with Zaffagnini's (1969) earlier finding of rudimentary hermaphroditism in this species, suggests that further study may provide evidence of additional examples of androdioecy among animals.

Acknowledgments

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US Natl. Mar Fish Serv. Fish Bull, 1985, 83(3): 219-234.

J. Ethol., 1987, 5(2): 145-160.

Thanks,
Peter Paras, Jr
Patent Examiner
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CM1, 12B03
703-308-8340

Sex Ratio: Adaptive Response to Population Fluctuations in Pandalid Shrimp

Abstract. *Pandalus jordani* is a protandrous (sequential) hermaphrodite. Populations show large year-to-year variation in age composition. In response to this variation, individuals alter the age at which they change sex. This response is predicted by a genetic model that assumes that an individual shrimp maximizes its genetic contribution to the next generation.

In this report we consider the problem of how natural selection operates on an organism's ability to either choose its sex or to alter the sex ratio among its progeny in response to environmental influences [environmental sex determination (ESD)]. Trivers and Willard (1) and Chewyreu (2) were among the first to realize that some environmental conditions favor a female's overproducing sons or daughters. In the Trivers-Willard model, the reproductive success (RS) of a son or a daughter is assumed to be closely related to the physiological condition of the mother. Applied to mammals, their model further assumes (i) both sons and daughters benefit in terms of RS when reared by a mother in good physiological condition, although (ii) a son benefits more than a daughter. They then concluded that natural selection favors females in good condition to overproduce sons and those in poorer condition to overproduce daughters. Some data from mammals may support this prediction (1). The key concept is that the sex gains more than the other (in terms of RS) when reared by a female in good condition.

This concept has recently been generalized to include sex expression in hermaphrodites and in dioecious species (with labile sex) in which an individual may become a male or a female depend-

ing upon the environment in which it finds itself (3). Some environmental conditions favor the RS of males, some that of females. Much evidence supports the prediction that hermaphrodites alter the allocation of resources to either male or female function depending on environmental conditions (3); that evidence has been interpreted in terms of why one sex benefits relatively more in a particular environment.

We have developed a genetic model for ESD in a dioecious species. The model makes some precise predictions, which we have tested with a protandrous hermaphrodite. (The "dioecy" assumption proves to be unimportant.)

Suppose that, because of spatial variation in food supply, members of a species (with ESD) find themselves at the time of breeding divided into big and small individuals. Sex is not yet determined. The questions of interest are (i) what fraction of the big individuals should reproduce as males? and (ii) what fraction of the small individuals should reproduce as females? In order to answer these, we must specify the consequences, in terms of individual RS, of being a male or a female of a given size.

Let P equal the proportion of breeders that are small. Further, let an individual which is big and reproducing as a female have an egg count of W_2 relative to a

small individual as a female. Big individuals reproducing as males will be given a fertility $f W_1$ relative to a small individual as a male.

Consider a very large population (of size N) with the characteristics that the proportion r_1 of the small individuals reproduce as males, while the proportion r_2 of the big individuals are males. If we define fitness to be the probability that an individual passes its genes to a zygote formed during the current breeding season (4-7), the fitness of a small individual (W_s), who reproduces as a male with some other probability (\hat{r}_1 , where $\hat{r}_1 \neq r_1$), may be written as:

$$W_s = \frac{1}{N} \left[\frac{\hat{r}_1}{P r_1 + r_2 W_1 (1 - P)} + \frac{1 - \hat{r}_1}{P(1 - r_1) + W_2 (1 - P)(1 - r_2)} \right] \quad (1)$$

$$W_s = \frac{1}{N} \left(\frac{\hat{r}_1}{M} + \frac{1 - \hat{r}_1}{F} \right)$$

The fitness of a big individual (W_b) who alters the probability (with which it reproduces as a male) to \hat{r}_2 (again $\hat{r}_2 \neq r_2$) is similarly defined:

$$W_b = \frac{1}{N} \left(\frac{\hat{r}_2 W_1}{M} + \frac{W_2 (1 - \hat{r}_2)}{F} \right) \quad (2)$$

If the situation is to be evolutionarily stable, it must be that an individual cannot increase its own fitness by altering the probability with which it becomes a male (or a female) away from the respective population values (6) (that is, W_s and W_b do not increase as \hat{r}_1 and \hat{r}_2 are altered from r_1 and r_2). The game now becomes to find r_1 and r_2 which satisfy this condition. The r_1 and r_2 we seek are those which maximize the product ($M \times F$). Although a formal proof is possible (8), we indicate here an intuitive justification for this result.

First, differentiate Eqs. 1 and 2 with respect to \hat{r}_1 and \hat{r}_2 , respectively. These have the form:

$$\partial W_s / \partial \hat{r}_1 \propto \frac{\partial H}{\partial r_1} \quad (3)$$

where $H = \ln M + \ln F$, and

$$\partial W_b / \partial \hat{r}_2 \propto \frac{\partial H}{\partial r_2} \quad (4)$$

As an example, consider Eq. 3. If H is maximized by setting $r_1 = 1$, then $\partial H / \partial r_1 \geq 0$, which implies that $\partial W_s / \partial \hat{r}_1 \geq 0$. An individual can do no better than also setting $\hat{r}_1 = 1$. The same holds if $r_1 = 0$ maximizes H ; here \hat{r}_1 should be set equal to zero to maximize W_s . If r_1 maximizes H by being set equal to some value between 0 and 1 (call it r_1^*), $\partial H / \partial r_1 = 0$. But then $\partial W_s / \partial \hat{r}_1 = 0$.

Table 1. Statistical analysis of Oregon data (14). Correlation coefficients were either the Pearson product moment coefficient (r) or the Spearman rank coefficient (r_s). Abbreviation: N.S., not significant.

Relation	Sample size	Regression	Correlation	P
Area 82-84				
First breeders (female)	8	$y = .62 - .54x$	$r = -.75$ $r_s = -.72$	<.05 <.05
Older breeders (male)	13 13	$y = .31 - .20x$	$r = -.84$ $r_s = -.78$	<.01 <.01
Area 86				
First breeders (female)	8*	$y = .57 - .29x$	$r = -.96$	<.01
	9†	$y = .44 - .16x$	$r = -.75$	<.05
	8*		$r_s = -.64$	<.05
Older breeders (male)	7 7	$y = .4 - .51x$	$r = -.73$ $r_s = -.38$	N.S. N.S.

*Fit without the point $x = 3.4$, $y = .14$. †Fit with the point $x = 3.4$, $y = .14$.

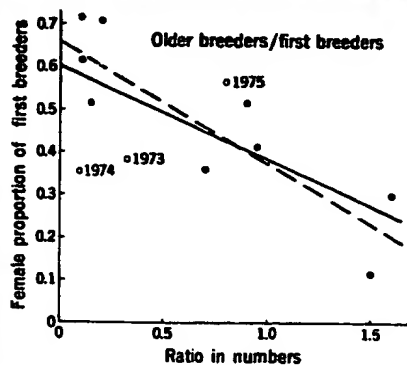


Fig. 1. *Pandalus jordani* in northern California for 12 years. Lines are the least-squares regression with (solid line) and without (dashed line) the years 1973 through 1975.

and selection is indifferent to whether an individual is a male or a female (so long as the group as a whole achieves r_1^*). A similar argument may be given for r_2 .

The maximization of $M \times F$ (provided $W_2 > W_1$) follows these rules:

$$1 - r_1 \begin{cases} = \frac{1}{2} \left[1 - W_2 \left(\frac{1-P}{P} \right) \right] & \text{if } W_2 > \frac{1-P}{P} \\ = 0 & \text{if } W_2 < \frac{1-P}{P} \end{cases} \quad (5)$$

and

$$r_2 \begin{cases} = \frac{1}{2} \left[1 - \frac{1}{W_1} \left(\frac{P}{1-P} \right) \right] & \text{if } W_1 > \frac{P}{1-P} \\ = 0 & \text{if } W_1 < \frac{P}{1-P} \end{cases} \quad (6)$$

This solution has the characteristics that (i) sex ratio within a size class depends on the frequency distribution of size classes, but (ii) more than 50 percent of the small shrimp should be males, while more than 50 percent of the big shrimp should be females, because females gain more RS by being big than males do (that is, $W_2 > W_1$). This corresponds to the Trivers-Willard RS assumption for mammals.

To test these predictions, we have used data from the shrimp *Pandalus jordani*, the major commercial shrimp off the northwest coast of the United States. It is a protandrous hermaphrodite (changes from male to female) that breeds once a year, in the fall. Most populations are short-lived, with at most three breeding age classes (9). The egg count of a shrimp reproducing as a female is positively related to body size (10), which increases with age. Because of large year-to-year variations in survival of the immature shrimp, the fraction of the population made up of shrimp breeding for the first time (small shrimp) varies greatly from year to year. Because most of the older breeders are second breed-

ers, we treat the problem as if two size classes are present. Provided that growth and death rates from the age of first breeding to the age of second breeding are not sex-specific (that they do not depend upon whether a shrimp breeding for the first time acts as a male or a female), natural selection should act on individual's choice of sex so that it maximizes its contribution to the zygotes formed in the current breeding season. The problem is then the same as the ESD problem just outlined; W_1 and W_2 are the relative fertilities of older shrimp, acting as males and females, respectively, and P is the fraction of the breeding population made up of first breeders. Being a sequential hermaphrodite makes no difference because the theory predicts that fewer than half of the smaller shrimp (first breeders) should be females and more than half of the bigger (older) shrimp should be females. Thus, the theory requires only that when the first breeders become 1 year older, more of

them change to females.

For three populations of *P. jordani*, we estimated the following for each of several years: (i) the fraction of breeders that were breeding for the first time (age 1.5 years), (ii) the fraction of first breeders that were females, and (iii) the fraction of older breeders that were males. Two data sources reflected the differences in the data available from

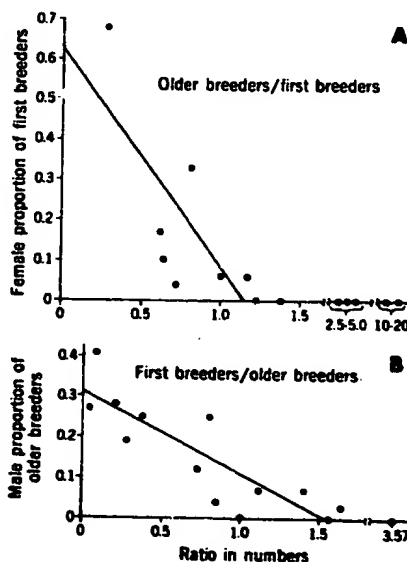


Fig. 2. *Pandalus jordani* in Oregon (fishing areas 82-84 (14)) for 14 years. (A) First breeders. (B) Older breeders.

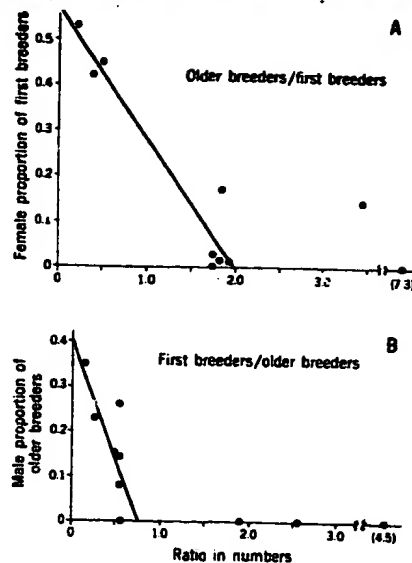


Fig. 3. *Pandalus jordani* in Oregon (fishing area 86 (14)) for 10 years. (A) First breeders. (B) Older breeders.

California and Oregon. For California, we judged the best data that from the fall research cruises of the California Department of Fish and Game. An extensive sampling program, designed specifically to estimate shrimp life history variables and abundance, has been carried out since 1964. We used estimates derived from those data from 1964 through 1975 (there was no cruise in 1976). Since the fishery may have suffered from over-exploitation in the early 1970's, we did the analysis both with and without the years 1973 through 1975. For Oregon, data were mostly derived from commercial fishery samples (11) (except for a few years when the Oregon Department of Fish and Wildlife research data were available). All data refer to the fall breeding population (for Oregon, September or later; for California, the dates of the fall research cruise). We separated year classes by examining frequency distributions of shrimp length, which were fairly distinct in these populations (9, 11). There are two consistent sources of bias in this analysis. (i) Shrimp fishing gear tends to select larger shrimp (9). (ii) Shrimp at the age of first breeding that act as females are slightly larger than same-aged shrimp that reproduce as males. Likewise, older breeders reproducing as males tend to be smaller than those breeding as females (9, 11). These sources of bias imply that (i) estimates of the ratio $(1 - P)/P$ will be inflated, reflecting the extent to which the respective size groups are caught (thus their representation in the samples), and (ii) estimates of r_1 and r_2 should be smaller than the true values, reflecting the dif-

ferent probabilities within an age class of being caught.

However, these biases should not alter the general shape of the predicted relations. The hypothesis has three parts. (i) If the proportion of first breeders acting as females (y variable) is plotted against the ratio (in numbers) of older to first-breeders (x variable), we expect a negative relation for small x values and no relation for large x values. (ii) If the proportion of older breeders acting as males is plotted against the ratio of first to older breeders, we expect the same shape of relation as given in (i). (iii) We expect to find some big males and some small females only if both of the relations given above have some y values at zero. These three predictions are a qualitative description of Eqs. 5 and 6.

For California, the proportion of first breeders acting as females is negatively correlated with the ratio of older breeders to first breeders (Fig. 1). Least-squares regression gives the fit $y = .60 - .20x$ ($r = .67$, $N = 12$, $P < .05$). However, recent years (1973 through 1975) show a relation different from the rest of the data. If only data before 1972 are used, the regression is considerably improved ($y = .68 - .3x$, $r = .87$, $N = 9$, $P < .05$).

Since the usual assumptions of regression and correlation are probably violated in these data, we also calculated a Spearman rank correlation coefficient (r_s). With all the data, $r_s = .51$, which just reaches the .05 significance level ($N = 12$). Again, recalculating r_s without the years 1973 through 1975 greatly improves the fit ($r_s = .85$, $N = 9$, $P < .01$). The theory also predicts that no older breeding shrimp should be males; none were found.

The two Oregon populations differ from the California one in that both show years in which some older individuals reproduced as males. The data support our hypothesis (Figs. 2 and 3) (Table 1). All but one regression (for points in the positive region) are significant, and, for these, the rank correlation is also significant.

Several other systems may be used to further test the model developed here. The situation discussed by Trivers and Willard (1) is one such case. If P is the fraction of the mothers in poor condition, W_1 the relative fitness of a son of a mother in good condition, and W_2 the relative fitness of a daughter of a mother in good condition, the same sex-ratio rules apply. The same rules may also apply to some solitary wasps and bees (2, 12) and to various orchids (13). Some orchids are

sexually labile, with individuals found in the bright sunlight mostly reproducing as females and those in the shade as males. Other organisms with ESD may be treated similarly.

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Newport 97365

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20 September 1977; revised 20 December 1977

5-Thio-D-Glucose Selectively Potentiates Hyperthermic Killing of Hypoxic Tumor Cells

Abstract. To investigate the mechanisms by which heat affects cancer cells, we used 5-thio-D-glucose, an inhibitor of glycolysis in HeLa S-3 cells, under aerobic and hypoxic conditions at temperatures ranging from 37° to 43°C. Drug alone or heat alone killed a minimum number of cells under aerobic or hypoxic conditions. Exposure to drug and hyperthermia selectively increased the number of cells killed under hypoxic conditions at temperatures as low as 40.5°C but had little effect on cells incubated under aerobic conditions. These results suggest that the glycolytic pathway is a primary site of hyperthermic damage leading to cell death.

It has been claimed repeatedly for more than a century that hyperthermia (temperatures above 40°C) can have a selective lethal effect on cancer cells. However, only sporadic attempts have been made to use these observations clinically, partly because of a poor understanding of the mechanism of enhanced thermal sensitivity of tumor cells, and partly because of the difficulty of selectively heating a chosen tumor volume at depth. Recently, more conclusive evidence of the action of heat on cancer cells both in vitro and in vivo has generated a renewed interest in this subject (1).

In cell culture studies (1), numerous cellular factors involved in the thermal response of tumor and normal cells have been identified. Thermal sensitivity is strongly dependent on such factors as cellular growth states, cell cycle phases, nutritional status, ambient oxygen concentration, and intra- and extracellular

pH (2). Of importance to clinical cancer therapy is the finding that hypoxic cells are sensitive to heat. Indeed, several laboratories reported hypoxic cells were far more sensitive to heat than cells cultured under aerobic conditions, though caution must be exercised in interpreting these data because the experimental induction of hypoxia is invariably associated with changes in the nutritional status of cultured cells, for example, the glucose concentration and extra- and intracellular acidity. When tumor cells are cultured at 42° to 43°C, their respiratory activity, including anaerobic glycolysis, is significantly reduced (3). In contrast, in normal cells cultured at the same temperature the respiratory activity is not as severely affected. The importance of anaerobic glycolysis in the pathogenesis of the cancer cell was discussed by Warburg (4). His original observation was that when both normal and malignant tissue slices were incubated in a medium containing

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Fishery Bulletin

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LIFE HISTORY CHARACTERISTICS OF *PANDALUS MONTAGUI* AND *DICHELOPANDALUS LEPTOCERUS* IN PENOBSCOT BAY, MAINE

DAVID K. STEVENSON¹ AND FRAN PIERCE²

ABSTRACT

A number of life history characteristics of two species of pandalid shrimp from Penobscot Bay, ME, were inferred from length-frequency and relative abundance data collected on five occasions during a bottom trawl survey in 1980-81. *Pandalus montagui* is a sequential hermaphrodite. Sex transition occurs throughout the year, but most transitional individuals were observed in late March. Most individuals change sex shortly before or after reaching age 2, but some do so either a year earlier or a year later. Ovigerous females were observed from late November through January; eggs are apparently produced during the second, third, and fourth years. Fifteen percent of the 0 age-group caught in the fall of 1980 were females which may never have functioned as males. Growth was rapid in the spring and summer and negligible in the late fall and winter. Females which changed sex at age 1 were larger than females which changed sex a year later. *Dichelopandalus leptocerus* is not hermaphroditic. Ovigerous females were collected primarily in late November and early December. Some females produce eggs during their first and second years, but most do so only during their second year. None of the females caught during this study appeared to be older than age 2; a few large males remained in the population during their third year of life. Females of both species were larger than males of the same age-group, a distinction which was attributed to differences in growth rate and, for *P. montagui*, was associated with earlier sex transition. Larger shrimp of both species migrated down the Bay into deeper water as the winter progressed.

The Pandalidae are a family of boreal, subarctic shrimp composed of 2 genera and about 20 species. Four species (*Pandalus borealis*, *P. montagui*, *P. propinquus*, and *Dichelopandalus leptocerus*) are common in offshore waters of the Gulf of Maine (Wigley 1960). Of these, *P. borealis* is the largest, reaching a maximum total length of 17-18 cm (7 in), and has been the object of a directed winter fishery in coastal waters of the Gulf of Maine since the late 1930s (Scattergood 1952) and in coastal and offshore waters since 1958³. This species is also exploited commercially on the west coast of North America, in the Canadian Maritime Provinces, on the west coast of Greenland, in the Norwegian and North Seas, and in the northwest Pacific (Balsiger 1981).

Pandalus montagui and *D. leptocerus* are smaller species (maximum length 10 cm or 4 in), which are harvested incidentally with *P. borealis* in the Gulf of

Maine, but have little or no market value because of their size. *Pandalus montagui* is also harvested as an incidental species in the Gulf of St. Lawrence (Balsiger 1981), and for many years was the object of several localized commercial beam trawl fisheries in the southern North Sea and in Morecambe Bay, northwest England, until declining stock sizes led to the demise of the fisheries in the Thames estuary (described by Mistakidis 1957) and Morecambe Bay in the 1950s and 1960s. Warren (1973) described a fishery for *P. montagui* in the Wash on the east coast of England which was still active in the early 1970s. *Pandalus propinquus* is also smaller than *P. borealis* and is generally restricted to deeper water (165-330 m in New England waters according to Wigley 1960); consequently it is rarely taken in Gulf of Maine commercial catches.

Pandalus montagui is differentiated taxonomically into two subspecies: *P. montagui tridens* in the North Pacific and *P. montagui montagui* in the North Atlantic from the Arctic south to the British Isles and Cape Cod (Simpson et al. 1970) or Rhode Island (Rathbun 1929). According to Simpson et al. *P. montagui montagui* is found in estuaries, coastal waters, and offshore in depths of 5 to over 700 m, but is more common in shallow waters (20-90 m); at depths > 90 m it is gradually replaced by *P. borealis*.

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²Maine Department of Marine Resources, West Boothbay Harbor, ME 04575.

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METHODS

Squires (1968) reported that *P. borealis* occurred together with *P. montagui* in depths < 200 m in the Gulf of St. Lawrence and southwest of Newfoundland, but at depths between 200 and 300 m with a smooth detritus bottom and temperatures of 4°-6°C, only *P. borealis* were caught; in colder temperatures (-1° to 3°C) in this same depth range, *P. montagui* were more abundant. *Pandalus montagui* was described as a more eurythermal and eurybathic species than *P. borealis*. Of all the pandalids in the northwest Atlantic, *P. montagui* is the only one which inhabits colder Arctic waters < 1.5°C (Squires 1966). The Atlantic subspecies of *P. montagui* has been the subject of several biological studies (Mistakidis 1957; Allen 1963; Couture and Trudel 1969a, b).

Dichelopandalus leptocerus is distributed in the northwest Atlantic from Newfoundland to North Carolina (Rathbun 1929). It has not been reported from the northeast Atlantic and is rare in the northern Pacific (Squires 1966). During a November 1956 bottom trawl survey in New England waters, *D. leptocerus* was much more widely distributed than *P. montagui* or *P. borealis* (Wigley 1960). *Dichelopandalus leptocerus* was also found over a broad depth range (33-340 m), but was common between 35 and 145 m and at temperatures (in November) of 5°-20°C, whereas *P. montagui* occurred primarily between 70 and 135 m and at temperatures of 6°-10°C. *Dichelopandalus leptocerus* was also collected in areas where bottom sediments contained low, medium, and high quantities of organic matter, whereas *P. montagui* appeared to be associated with sediments with relatively low organic content. Thus, in several ways, *D. leptocerus* appears to have less restricted habitat requirements than *P. montagui* (or *P. borealis*). No detailed biological studies of *D. leptocerus* have been published.

The Maine Department of Marine Resources conducted an exploratory bottom trawl survey to determine the abundance and distribution of pandalid shrimp populations in Penobscot Bay (Figure. 1) during 1980-81. During the course of this survey, biological data were collected from about 10,000 shrimp. The objective of this paper is to describe important life history characteristics of *P. montagui* and *D. leptocerus* in Penobscot Bay (the Bay); these include breeding seasons, female sizes and ages at maturity, sex transition, growth, longevity, and migratory behavior. Aspects of the life cycle and reproductive biology of each species were examined as functions of time of year, depth, and location within the Bay.

The survey was conducted over the course of a 12-mo period from late November 1980 to early October 1981. Samples were collected during five distinct periods of time at 19 different stations located from Cape Jellison in the northern end of Penobscot Bay to Mark Island, a distance of about 37 km (23 mi) (Fig. 1). Stations were established at depths ranging from 12 m (40 ft) to 84 m (280 ft) and were located in areas of trawlable bottom. Since a primary objective of the survey was to stimulate commercial shrimp fishing, no attempt was made to randomly select station locations, depths, or sampling times. Attempts were made, however, to return to each station as often as possible so as to determine the seasonal variation in the relative abundance of different sexes, reproductive stages, and size groups of each species at individual locations over the course of the year. Adjacent, well-defined, length groups were assumed to represent successive age-groups.

A total of 45 successful tows (i.e., tows that were not aborted because of bottom obstructions, damage to the trawl, or gear malfunction) were made during the entire survey. Of these, 37 tows which could be assigned to a specific area, depth range, and sampling period were selected for data analysis. Area 1 was defined as the upper Bay, area 2 as west of Islesboro, area 3 as south of Islesboro, and area 4 as east of Islesboro; depth ranges were defined as shallow (12-25 m), moderate (25-50 m), and deep (50-85 m) (Table 1). The distributions of sampling effort between stations by sampling period, area, and depth range are shown in Table 2. No data were

TABLE 1.—Definitions of coded sampling periods, areas, and depth ranges, 1980-81 Penobscot Bay shrimp survey.

Sampling periods	
1	20 November-2 December 1980
2	21-29 January 1981
3	24-31 March 1981
4	16 July-18 September 1981
5	5-6 October 1981
Areas	
1	Upper Bay: stations 2, 3, 4, 6, 18
2	West of Islesboro: stations 1, 5, 9, 10, 14
3	South of Islesboro: stations 7, 8, 12, 15, 16, 17, 19
4	East of Islesboro: stations 11, 13
Depth ranges	
1	12-15 m (shallow)
2	25-50 m (moderate)
3	50-85 m (deep)

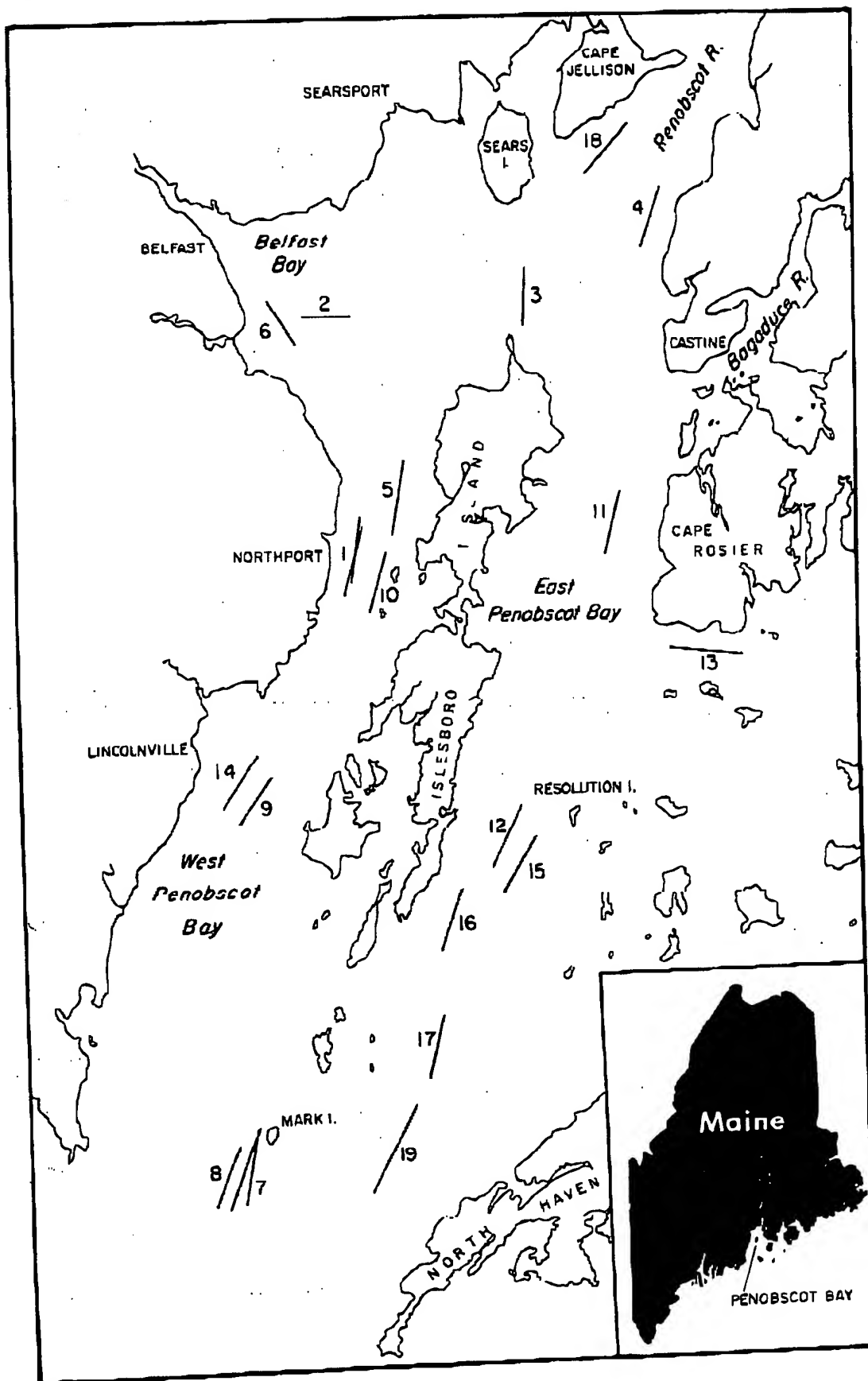


FIGURE 1. - Map of Penobscot Bay, ME, showing bottom trawl stations visited during 1980-81 survey.

TABLE 2.—Distribution of sampling effort (number of tows per station) by sampling period, area, and depth range, 1980-81 Penobscot Bay shrimp survey.¹

Station	Sampling period					Area				Depth		
	1	2	3	4	5	1	2	3	4	1	2	3
1		2	1	6		9				9		
3		1		1		2				2		
4		1				1				1		
5		1					1				1	
6		1				1				1		
7			1	1	1			3				3
8		1					1					1
9		1		1		2				1	1	
10		1	1	3		5				5		
11			1		1				2	1	1	
12			1					1				1
13			1						1			1
15			1					1				1
16			1	1	1			3				3
17			1					1				1
18			2			2				2		
19				1					1			1
Totals	9	10	9	6	3	6	17	11	3	6	17	14

¹See Table 1 for definitions of coded sampling periods, areas, and depth ranges.

available from stations 2 and 14. Trawling operations were limited to the area between Northport and Islesboro in the summer of 1981, since the only vessel available at that time was not equipped to work elsewhere in the Bay.

The trawl used was a semiballoon shrimp try-net with a 7.6 m (25 ft) headrope and 9.5 m (31 ft) footrope with no rollers; mesh size was 38 mm (1½ in) in the body and 31 mm (1¼ in) in the cod end. The trawl was also equipped with a 12.5 mm (½ in) liner. The net was rigged on 1.2 m (4 ft) legs with ark floats on the headrope and 2/0 chain on the footrope. The trawl doors were hardwood, 76 × 41 cm (30 × 16 in), with iron bracings and a wide shoe. The net was fished on a single trawl wire attached to a 30.5 m (100 ft) wire net bridle. All tows lasted 30 min and were made at speeds varying from 1.5 to 2.5 kn. Loran bearings were recorded at the beginning and end of each tow, and depth was recorded either as a single reading or at the beginning and end of each tow. Location and depth could not be determined in the summer since the vessel used then did not have sonar or navigational equipment. Although three different vessels were used during the course of the survey, the gear was identical and was fished the same way during the entire survey.

If catches were small (under 1 kg), the entire catch was generally brought to the laboratory and frozen for later analysis; otherwise, the catch was subsampled aboard the vessel. In some cases, large samples were further subsampled in the laboratory

after they were thawed. Inasmuch as was possible, all samples and subsamples were randomly selected. Samples (or subsamples) of 200-900 g were sorted (after removing extraneous "trash") by species according to morphological characteristics described by Rathbun (1929). Biological data were compiled for a total of 7,259 *D. leptocerus* and 2,475 *P. montagui*; numbers of *P. borealis* were inadequate for data analysis. Each individual shrimp was sexed (male, female, or transitional) using external morphological characteristics for the genus *Pandalus* originally described by Wollebaek (1908), Berkeley (1930), Jägersten (1936), and Leloup (1936) and summarized by Mistakidis (1957). The females were further grouped as ovigerous or non-ovigerous depending on whether or not the eggs had "dropped" and were being carried on the pleopods; the non-ovigerous females were further subdivided into two groups—those which had never carried eggs before and those which had—based on the presence or absence of sternal spines. This characteristic of non-ovigerous females was originally described by McCrary (1971) for three pandalid species (*Pandalus borealis*, *P. goniurus*, and *P. hypsinotus*) in Alaska. Stage I females were defined as those which had not carried eggs before and Stage II females as those which had; there was no way to distinguish between females which had carried eggs only once before and those which had carried eggs more than once. Carapace lengths were measured between the eye socket and posterior dorsal edge of the carapace and recorded to the nearest 0.1 mm.

For each species, the numbers and lengths of shrimp in each biological category (sex, with or without eggs, Stage I or II) were compiled by sampling period, geographic area, and depth range. Length frequencies were expressed as numbers of shrimp per 0.5 mm dorsal carapace length. Since nearly all of the samples collected in areas 1-3 were also collected in specific depth ranges (i.e., all 6 samples from area 1 were from shallow water, 16/17 samples from area 2 were from moderate depths, and all samples from area 3 were from deep water), length frequencies were presented for appropriate area/depth combinations. Length-frequency data for *P. montagui* collected in October 1981 were not presented since so few individuals were captured.

RESULTS AND DISCUSSION

Breeding Seasons and Female Sizes (Ages) at Maturity⁴

Nearly all the ovigerous female *D. leptocerus* were

caught in November-December 1980 (Table 3), although a few remained in January and March. It was therefore apparent that most eggs hatched during a relatively short period of time in late December and early January. Although only the larger size group was carrying eggs (Fig. 2D), the presence of a

were reported for the same populations as late February through April with peak activity in April. According to Couture and Trudel (1969b), ovigerous females were observed in Grand-Rivière, Quebec, beginning in July and accounted for the greatest percentage of the population in October (no samples

TABLE 3.—Percent total number of male and female *Dichelopandalus leptocerus* collected at all locations and depths in Penobscot Bay during five sampling periods in 1980-81. (Females are categorized by reproductive stage.)

Sex/Stage	11/20-12/2 1980	1/21-1/29 1981	3/24-3/31 1981	7/16-9/18 1981	10/5-10/6 1981	Total
Total males	49.7	65.3	59.4	47.6	53.0	53.2
Females/Stage I	32.0	33.5	37.8	50.2	45.3	38.8
Females/Stage II	1.4	0.3	1.5	2.2	1.5	1.5
Total						
non-ovigerous females	33.4	33.7	39.3	52.4	46.8	40.2
Ovigerous females	16.8	1.0	1.3	0.0	0.2	6.6
Total females	50.3	34.7	40.6	52.4	47.0	46.8
Total no. individuals	2,694	729	1,151	1,107	1,577	7,259

few Stage II non-ovigerous females in roughly the same size range (Fig. 2C) indicated that some females produced eggs a year earlier as well. It could not be determined from the samples collected during this study whether the younger females spawned earlier or later than the older group. The fact that so few Stage II females were captured in the Bay at any time of year indicated that most of the spawning population was made up of first time spawners. Ovigerous females were collected at all depths and in all areas (Tables 4, 5), but made up a greater percentage of the catch at moderate depths in area 2.

Nearly all of the ovigerous female *P. montagui* were collected in November-December and late January (Table 6); the fact that 50% of the females in late January were still ovigerous suggests that eggs hatched over a more prolonged period than was true for *D. leptocerus*, possibly from November at least through February. Females belonging to two North Sea *P. montagui* populations were reported to carry eggs primarily between November and February (Mistakidis 1957; Allen 1963), although ovigerous females were observed from mid-October to April in the Thames estuary by Mistakidis. Hatching times

*Since no internal sexual characteristics (such as oocyte development) were examined in this study, the breeding season was defined as the period of time when ovigerous females were observed and sizes (ages) at maturity as the sizes (ages) when females produce eggs. No comparable information for males (i.e., mating times or sizes (ages) at maturity) was available. As used in this paper, the breeding season was, strictly speaking, the period of time between spawning and hatching when eggs were incubated.

TABLE 4.—Percent total number of male and female *Dichelopandalus leptocerus* collected at all depths and times of year in four areas in Penobscot Bay during 1980-81. (Females are categorized by reproductive stage.)

Sex/Stage	Area 1	Area 2	Area 3	Area 4	Total
Total males	54.4	49.3	57.4	70.6	53.0
Females/Stage I	42.2	39.7	36.7	26.1	38.9
Females/Stage II	1.0	1.8	1.1	1.1	1.4
Total					
non-ovigerous females	43.2	41.5	37.8	27.2	40.3
Ovigerous females	2.4	9.2	4.8	2.2	6.6
Total females	45.6	50.7	42.6	29.4	47.0
Total no. individuals	1,047	3,626	2,419	92	7,184

TABLE 5.—Percent total number of male and female *Dichelopandalus leptocerus* collected in all areas and times of year by depth range in Penobscot Bay during 1980-81. (Females are categorized by reproductive stage.)

Sex/Stage	Shallow (12-25 m)	Moderate (25-50 m)	Deep (50-85 m)	Total
Total males	54.4	47.7	60.3	53.3
Females/Stage I	41.5	40.4	32.4	37.6
Females/Stage II	1.1	2.0	0.9	1.4
Total				
non-ovigerous females	42.6	42.4	33.3	39.0
Ovigerous females	3.0	9.9	6.4	7.7
Total females	45.6	52.3	39.7	46.7
Total no. individuals	812	3,015	2,299	6,126

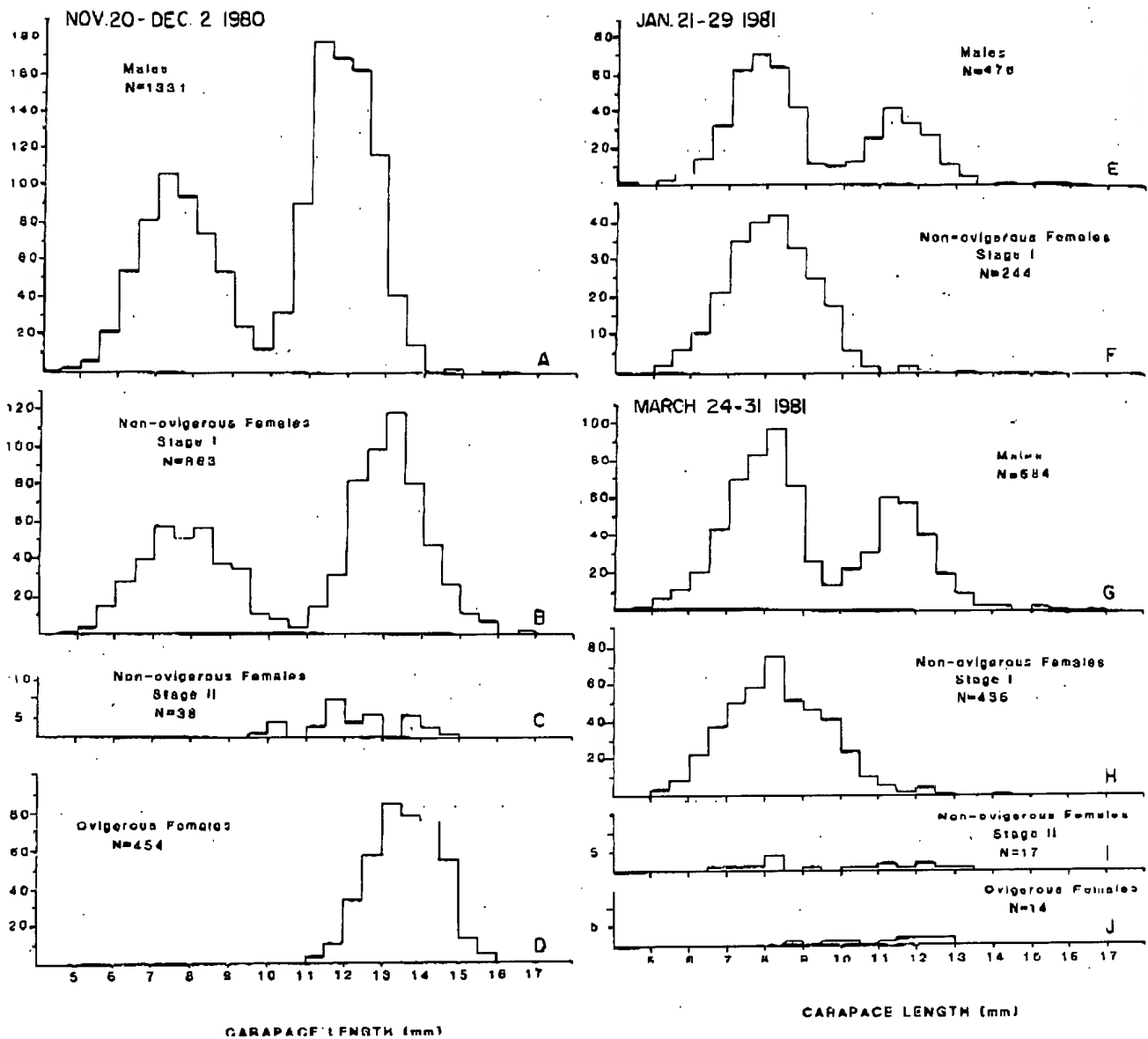
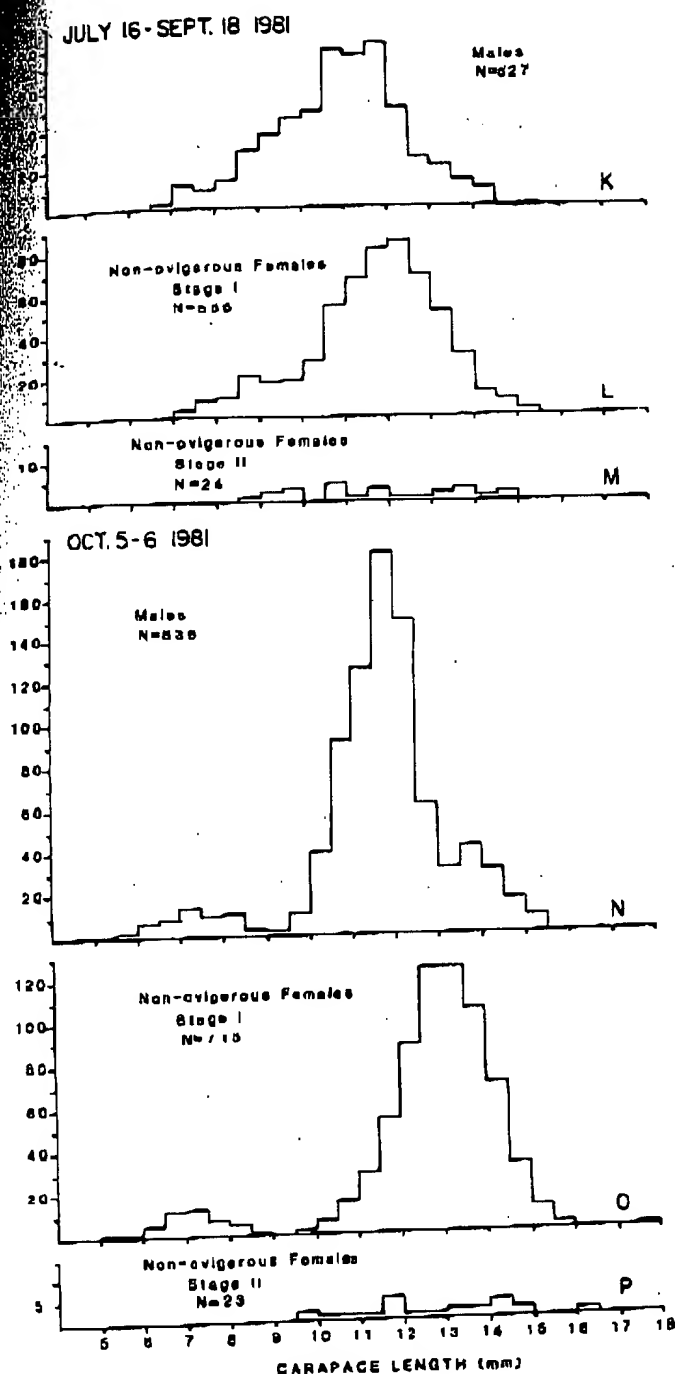


FIGURE 2.—*Dichelopandanus leptocerus* numerical length-frequency distributions by sex and reproductive stage.

TABLE 6.—Percent total number of male, transitional, and female *Pandalus montagui* collected at all locations and depths in Penobscot Bay during five sampling periods in 1980-81. (Females are categorized by reproductive stage.)

Sex/Stage	11/20-12/2 1980	1/21-1/29 1981	3/24-3/31 1981	7/16-9/18 1981	10/5-10/6 1981	Total
Total males	73.2	85.2	68.4	37.3	34.3	68.9
Transitionals	0.3	2.5	15.4	5.7	1.5	6.9
Females/Stage I	7.8	6.1	15.9	50.4	61.2	18.2
Females/Stage II	0.3	0.0	0.0	6.7	3.0	1.2
Total						
non-ovigerous females	8.1	6.1	15.9	57.0	64.2	19.4
Ovigerous females	18.4	6.2	0.4	0.0	0.0	4.8
Total females	26.5	12.3	16.2	57.0	64.2	24.2
Total no. individuals	332	871	800	405	67	2,475



were collected between November and April). A few females were still carrying eggs the following June. Hatching began in the winter and continued through June. *Pandalus montagui* populations studied in Penobscot Bay and Grand-Rivière spawned primarily in their second and third years. Females in the North Sea, on the other hand, were fully mature during their first year (Mistakidis 1957; Allen 1963), but there was no evidence that individual age-groups spawned more often there than at Grand-Rivière or in Penobscot Bay.

At least two age-groups of ovigerous *P. montagui* were apparent in the winter samples (Fig. 3C, G); whereas only a single age-group of ovigerous *D. leptocerus* was observed (Fig. 2D). Length-frequency data suggested that older female *P. montagui* (age 2+) spawned before younger females (age 1); 57% of the ovigerous females collected in November-December were > 15 mm CL (Fig. 3C), whereas only 30% remained in the same size group in late January (Fig. 3G). Sample sizes were much too small, however, to clearly indicate how many spawning age-groups were present or whether older females spawned earlier than younger ones. Earlier completion of spawning by older females was reported by Mistakidis (1957) in the Thames estuary. In Penobscot Bay, the relative abundance of ovigerous females was higher in moderate and deep waters and in areas 2, 3, and 4 (Tables 5, 6).

The capture of a single 10 mm CL ovigerous female *P. montagui* in January (Fig. 3G) indicates that a few females mature and reproduce during their first year. This shrimp could have started life as a female or could have changed sex in the first year and therefore never functioned sexually as a male. Mistakidis (1957) reported that some 0 age-group males in the Thames estuary changed sex and functioned as females during their first year. Some individuals in both the Thames estuary and Northumberland began life as females and matured in their first year (Mistakidis 1957; Allen 1963).

Sex Transition

Unlike most other Pandalid species, the Penobscot Bay population of *D. leptocerus* was not hermaphroditic. Not a single transitional individual was identified in the over 7,000 shrimp which were examined. Furthermore, males and females recruited to the > 5 mm CL population in October of their first year in nearly equal numbers (Fig. 2N, P). The ratio of males to females for the entire survey period was 58:47 (Table 3). *Pandalus montagui*, on the other hand, is a protandric (sequential) hermaphrodite. Nearly 7% of the 2,475 individuals examined exhibited external morphological characteristics typical of transitional *P. borealis* (Allen 1959). The sex ratio was 69% males to 7% transitionals to 24% females (Table 6). Although *P. montagui* is clearly protandric, some individuals in Penobscot Bay either begin life as females or assume external female characteristics by late November of their first year.⁵ These

⁵Individuals of protandric pandalid shrimp species which begin life as females are referred to as primary females; those which change

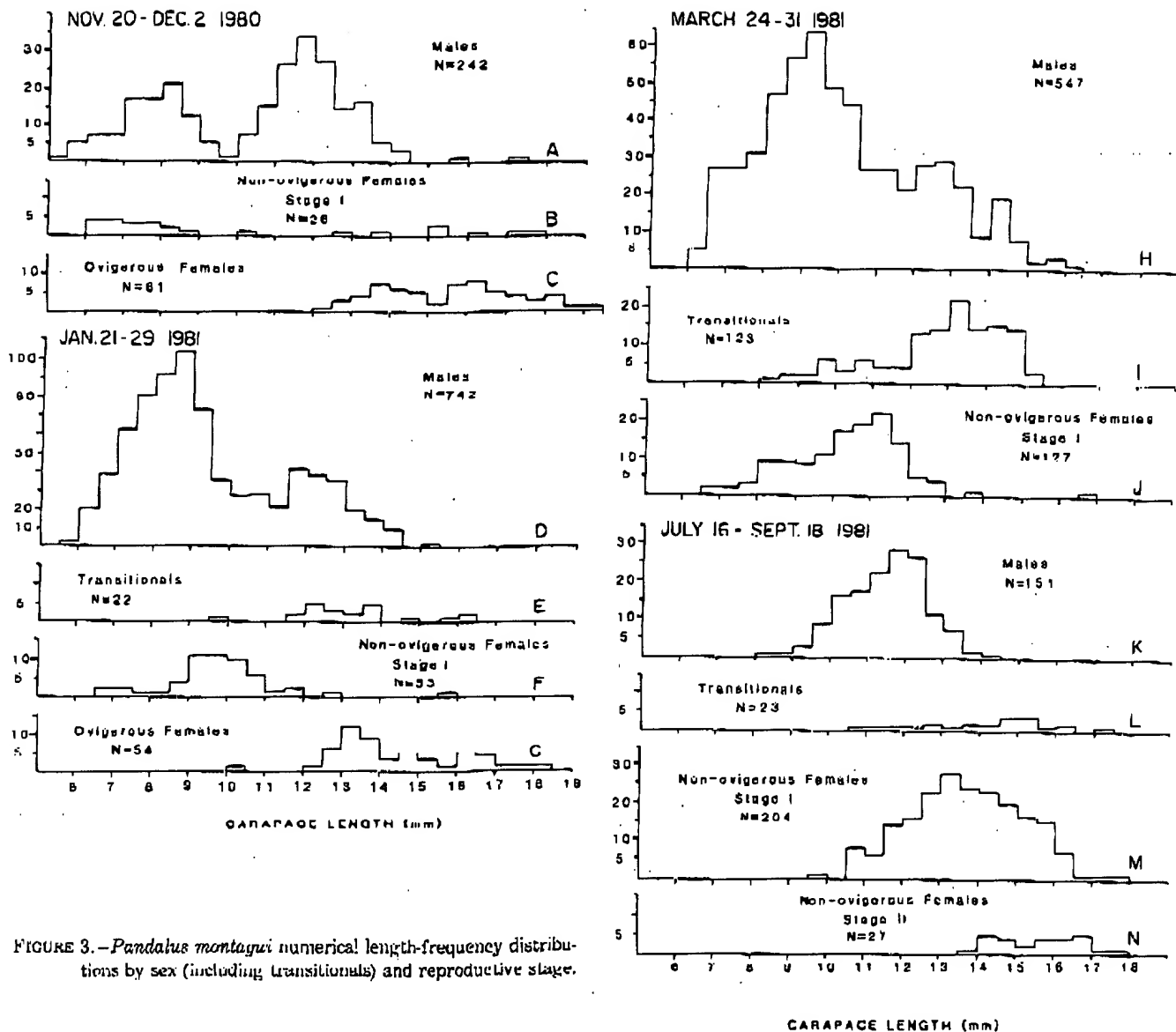


FIGURE 3.—*Pandanus montagui* numerical length-frequency distributions by sex (including transitionals) and reproductive stage.

females were obvious as a distinct size-group in the November-December length-frequency data at 6-9 mm CL (Fig. 3B). At this time, these females made up 15% of the newly recruiting 0 age-group. The relative abundance of 0 age-group females was considerably higher in the North Sea: 29-37% of most samples in the Thames (Mistakidis 1957) and about 50% in Northumberland (Allen 1963). On the

sex in their first year following the repression of male sex characteristics and never function as males are called secondary females; and those which function first as males and then change sex are called hermaphroditic females (Mistakidis 1957). Since no distinction could be made in this study between the three types of female *P. montagui*, we have avoided the use of this terminology altogether and simply distinguish between individuals which remained as males during their first year, those which were females when they were first captured in November-December of their first year, and those which apparently changed sex during their first winter.

other hand, only 11.2% of the age-1 *P. montagui* collected in May 1965 in Grand-Rivière were females (Couture and Trudel 1969b).

Size (Age) at Sex Transition

Transitional *P. montagui* were collected during all five sampling periods, but were most abundant in late March (Table 6) following the end of the breeding period. Sex transition apparently began in January, peaked in late March, and continued through the summer and early fall, reaching a minimum in late November. The rapid decline in the relative abundance of males after January 1981 and the accompanying increase in females after March (Table 6) indicated that shrimp, which functioned as males in the previous breeding season and became

females prior to the subsequent breeding season, assumed external female characteristics during the winter and spring. The appearance of a distinct size-group of 9-11 mm CL females in late January (Fig. 3F) which was not present 2 mo earlier (Fig. 3B), suggests that transition was well underway by late January. The two size-groups of non-ovigerous females in January and March (Fig. 3I) were assumed to belong to the same age-group, the smaller females being those which did not function as males in their first year (they may have started life as females) and the larger females being those which were still males in November-December (Fig. 3A). Transitional shrimp made up a larger percentage of samples collected in moderate and deep waters and in areas 3 and 4 (Tables 7, 8).

Transition of the younger age-group which was first captured in November-December (Fig. 3A) was incomplete since a great many shrimp remained as males for another entire year before undergoing

transition during their third spring (at age 2). Given the fact that a few male and transitional shrimp > 15 mm CL were sampled at various times of year, the possibility that a few individuals do not change sex until their fourth year (age 3) could not be ruled out. Even though the transition of younger (age 1) shrimp in the spring was incomplete, a sizable number of non-ovigerous Stage I shrimp which completed transition in their first year were collected in March (Fig. 3J). These new females were considerably larger (by about 2 mm CL) than their male counterparts, suggesting that it was the larger, faster growing, individuals which underwent transition at age 1. Allen (1963) also reported that the largest 0 age-group males changed sex first.

Older shrimp which changed sex in their second year had not yet appeared as females in March (Fig. 3J), suggesting that sex transition in older shrimp was delayed; it may also have been less rapid, particularly since growth was considerably reduced after the first year. Earlier studies of *P. montagui* indicated that sex transition in two locations in the North Sea persisted for most or all of the year. Mistakidis (1957) noted that sex transition occurred from May to December in the Thames estuary whereas Allen (1963) collected transitional individuals throughout the year in Northumberland, but primarily in June. The timing of minimal sex transition in Penobscot Bay and Northumberland was identical (November-December); Allen (1963) reported that this was when males were sexually active. Couture and Trudel (1969b) reported that most sex transition occurred in October at Grand-Rivière; a few transitionals were collected in July and August but none in May and June. Sex transition in the North Sea, as reported by Mistakidis (1957) and Allen (1963), was accelerated in comparison with Penobscot Bay; some individuals changed sex in their first year, but most did so in their second year. Ages at sex transition at Grand-Rivière, on the other hand, were the same as in Penobscot Bay, i.e., some in their second year, most in their third year, and some in their fourth year.

TABLE 7.—Percent total number of male, transitional, and female *Pandalus montagui* collected at all depths and times of year in four areas in Penobscot Bay during 1980-81. (Females are categorized by reproductive stage.)

Sex/Stage	Area 1	Area 2	Area 3	Area 4	Total
Total males	82.3	83.4	67.1	77.9	68.1
Transitionals	1.6	3.0	11.5	12.3	7.1
Females/Stage I	14.0	25.4	16.8	5.5	18.8
Females/Stage II	0.0	2.6	0.4	0.0	1.3
Total non-ovigerous females	14.0	28.0	17.2	5.5	20.0
Ovigerous females	2.2	5.7	4.2	4.3	4.7
Total females	16.1	33.6	21.4	9.8	24.8
Total no. individuals	106	1,037	827	326	2,376

TABLE 8.—Percent total number of male, transitional, and female *Pandalus montagui* collected in all areas and times of year by depth range in Penobscot Bay during 1980-81. (Females are categorized by reproductive stage.)

Sex/Stage	Shallow (12-25 m)	Moderate (25-50 m)	Deep (50-85 m)	Total
Total males	82.4	61.6	75.3	68.7
Transitionals	1.1	5.8	7.1	5.9
Females/Stage I	14.2	24.0	10.9	18.1
Females/Stage II	0.0	2.7	0.1	1.5
Total non-ovigerous females	14.2	26.6	11.1	19.6
Ovigerous females	2.3	5.9	6.6	5.8
Total females	16.5	32.6	17.6	25.4
Total no. individuals	176	1,013	732	1,921

Seasonal Changes in Size (Age) Composition

Two size-groups of male *D. leptocerus* were observed in the Penobscot Bay during the winter (Fig. 2A, E, G) and only one in the summer (Fig. 2K); similarly, female length-frequency distributions in November-December were bimodal (Fig. 2B), but a single size-group was dominant in the summer (Fig. 2L). Presumably, most of the older (age 2) males either die or migrate out of the upper Bay in the

spring since the younger age-group made up the entire population in the summer. Most of the females apparently spawn once and die after their eggs are hatched since very few older females were found in the winter. The presence of a few larger males in October (Fig. 2N) suggested that a few survive into their third fall and mate twice (or three times if they mature during their first year). Further evidence that most female mortality occurs following hatching while male mortality is delayed until later in the spring was indicated by the seasonal changes in relative abundance of males and females (Table 3): females decreased from 50 to 35% of the population between early December and late January while males decreased more slowly from 65% in January to 48% in the summer.

Age-2 *P. montagui* which were either in transition or were still males in March had mostly become females by the summer (although a few transitionals and large males still remained). The single large size-group of first-maturing Stage I females in the summer (Fig. 3M) presumably included age-1 females at a modal length of 13 mm CL and age-2 females at about 15 mm CL. At the same time, there appeared to be at least two size-groups of Stage II females in July-September (Fig. 3N) which had carried eggs the previous winter (Fig. 3C, G). Ovigerous females captured in November-December 1980 (Fig. 3C) presumably included first-time spawners at 12-15 mm CL and one or two groups of repeat spawners at 15-19 mm CL. Excluding the single female at 10 mm, two or three age-groups of ovigerous females were apparent in January (Fig. 2G). As indicated earlier, the relative abundance of the different age-groups in November-December and January showed that repeat spawners may have accounted for a larger percentage of the ovigerous females earlier in the winter.

Growth and Longevity

Male and female *D. leptocerus* which hatched in the winter of 1979-80 reached 6.0-8.5 mm CL by October of their first year (Fig. 2N, P) and grew relatively slowly during their first winter; by March they had reached 7-10 mm CL and the females were slightly larger than the males (Fig. 2G, H). This difference in size-at-age was also discernible in January (Fig. 2E, F). Growth was rapid during the spring of the second year prior to the beginning of the breeding season: males increased about 3 mm in carapace length by the summer while females increased by 4 mm (Fig. 2K, L). The difference in modal lengths between males and females had increased further by October

(Fig. 2N, P) as growth increased modal carapace lengths by an additional 0.5-1.0 mm for both sexes. Growth between early October and late November when ovigerous females were first sampled was negligible; if October 1981 data can be compared with November 1980 data. During this same short period of time the relative abundance of the younger, newly recruited, age-group (males and females) increased dramatically. (A reduction in somatic growth can be expected at a time of rapid egg development since female growth ceases once their eggs have "dropped" and they are unable to molt). This species reached a maximum observed size of 19 mm CL (not included in compiled length frequencies), but most individuals did not exceed 16 mm CL. Unless older individuals migrate completely out of Penobscot Bay and were therefore not sampled during this survey, the maximum lifespan of *D. leptocerus* in the Bay appears to be about 2 yr and 9 mo, although the bulk of the population apparently survives for only 2 yr.

The first evidence of newly recruited 0 age-group *P. montagui* was in November-December 1980 (Fig. 3A, B). Individuals which remained as males during their second year grew from about 7-9 mm CL in November-December of their first year to 8-10 mm CL in March (Fig. 3H) and 10-13 mm CL in their second summer (Fig. 3K). As was observed for *D. leptocerus*, the growth rate increased in the spring. Age-1 shrimp which became females during their second spring reached 10-12 mm CL in March (Fig. 3J) and 12-15 mm CL in the summer (Fig. 3M). Comparison of November-December 1980 and January 1981 data (Fig. 3C, G) with summer 1981 data suggested that growth of mature females in the fall was negligible; the same was true for the males. The maximum observed size was 19.5 mm CL; females as large as 17-18 mm CL were collected in the winter (Fig. 3C, G). These results suggested that *P. montagui* in Penobscot Bay normally spend 1-2 yr as males and as many as 8 yr as females. The maximum lifespan is probably 4 yr since shrimp that remain males for 2 yr do not complete sex transition until their third year and function as females in their third and fourth years.

Growth at Grand-Rivière was sufficiently slower that males there were 2-3 mm CL smaller by the end of their first year than they were in Penobscot Bay. This difference in growth could be a result of lower summer bottom temperatures in the Gulf of St. Lawrence. A temperature range of -1°C (in May) to 3°C (in October) was reported in 54 m at Grand-Rivière in 1965 (Couture and Trudel 1969a). Temperatures recorded in lower Penobscot Bay during the same months of the year at 40-60 m were

considerably higher, i.e., 3°C west of Islesboro in May 1970⁶ and 11°-12°C at various stations in the lower Bay in August 1976⁷. In Penobscot Bay, *P. montagui* were smaller after their first year's growth than in the two North Sea locations (Mistakidis 1957; Allen 1963) but attained approximately the same size by the end of the second year. Males in the Thames estuary measured 10 mm average CL by November of their first year, and in Northumberland (at 40-60 m depth) they averaged 9.5 mm CL by October; transitionals reached 12.5 mm CL by November of their second year in both locations as compared with 11-13.5 mm CL males of the same age in Penobscot Bay (Fig. 3A), while females in Northumberland reached 14.8 mm CL by November of year 2 as compared with 13-15 mm CL at the same age in Penobscot Bay (Fig. 3C). One-year-old females in Northumberland averaged 10.8 mm CL in October.

Allen's (1963) explanation for this difference between male and female lengths-at-age was that shrimp which mature as females in their first year do so 3-4 wk after males of the same age-group; since growth virtually ceases in the fall and winter, the difference in length attained by the females in the first year is maintained into the third year of life. In Penobscot Bay, on the other hand, very few females mature in their first year; however, if males mature in their first year (this was not determined) and stop growing in the fall before the females, Allen's explanation might apply. It seems more likely that sex transition is a function of size, not age, and that the faster growing 0 age-group shrimp complete sex transition in their first year. Another possible explanation for the difference in size of females which change sex in their second and third years is that there may be two distinct periods of larval production and/or survival. Length-frequency data collected at two different periods during the winter (Fig. 3C, G) did suggest that older females may have spawned earlier than younger females. A 5-yr study of *P. borealis* in the Sheepscot River of Maine⁸ failed, however, to reveal any consistent bimodality in larval production during February-April even though at least two age-groups of ovigerous females are com-

monly observed in commercial catch samples⁹. We contend, therefore, that the most plausible explanation for differences in the sizes of shrimp which become females in their second and third years is a difference in growth rates, especially since a similar difference in size was observed between male and female *D. leptocerus* in which sex remains fixed throughout life.

Pandalus montagui which either began life as females or became females early in their first year were smaller than their male counterparts by November-December of their first year (Fig. 3A, B). It therefore seems probable that the smaller Stage I (6.5-8.5 mm CL) females captured in late January (Fig. 3F) did not grow as rapidly as the larger females in the same age-group which apparently completed sex transition in late December and early January or as shrimp which remained as males for the entire year (Fig. 3D). These differences in length-at-age between 0 age-group males and both groups of females were also evident in late March (Fig. 3H, J). The accelerated growth rate of 0 age-group individuals which changed sex during their first year contrasts with the reported faster growth of *P. montagui* in the North Sea and at Grand-Rivière which began life as females (Allen 1963; Couture and Trudel 1969b).

Winter Migration

During November-December 1980, younger male and non-ovigerous female *D. leptocerus* were predominant in shallow water in area 1 (Figs. 4A, 5A) while the older age group predominated in deeper water (Figs. 4B, C, 5B, C). By late January, the older females were no longer being caught, and the older males had disappeared completely from depths < 50 m (Fig. 4D, E), but accounted for about 50% of the males collected in deep water in area 3 (Fig. 4F). There were older males in areas 2 and 3 (moderate and deep water) in March (Fig. 4G, H). These results suggested that older male shrimp migrated down the Bay into deeper water as the winter progressed and as bottom water temperatures dropped from about 8°C in early December to 1°C in late February and early March in the upper Bay.¹⁰ Their disappearance from the catches,

⁶Muirhead, C. R., and J. H. Wartha. 1971. Temperature-salinity observations, Penobscot Bay, Maine, 1970. Oper. Data Rep. NOS DR-13. U.S. Dep. Commer., NOAA, Natl. Ocean Surv., Off. Mar. Surv. Maps, Oceanogr. Div., Descr. Oceanogr. Sect., Rockville, MD.

⁷Central Maine Power Co., unpublished data, courtesy Richard Birge, Environmental Studies Department, CMP, August, ME.

⁸Stickney, A. P. Environmental physiology of northern shrimp, *Pandalus borealis*. Maine Dep. Mar. Resour., West Boothbay Harbor, ME, Annu. Rep. 1981-82, 15 p.

⁹Diodati, P., S. H. Clark, D. McInnes, R. Tichko, and D. Sampson. 1983. Gulf of Maine northern shrimp stock status -1983. Northern Shrimp Technical Committee, November 1983, 9 p.

¹⁰Birge, R. P. 1982. Surface and bottom water temperatures, upper Penobscot Bay, Maine, March 1975 - December 1981. Central Maine Power Co., Environmental Studies Department, Report SI-82-3, 45 p.

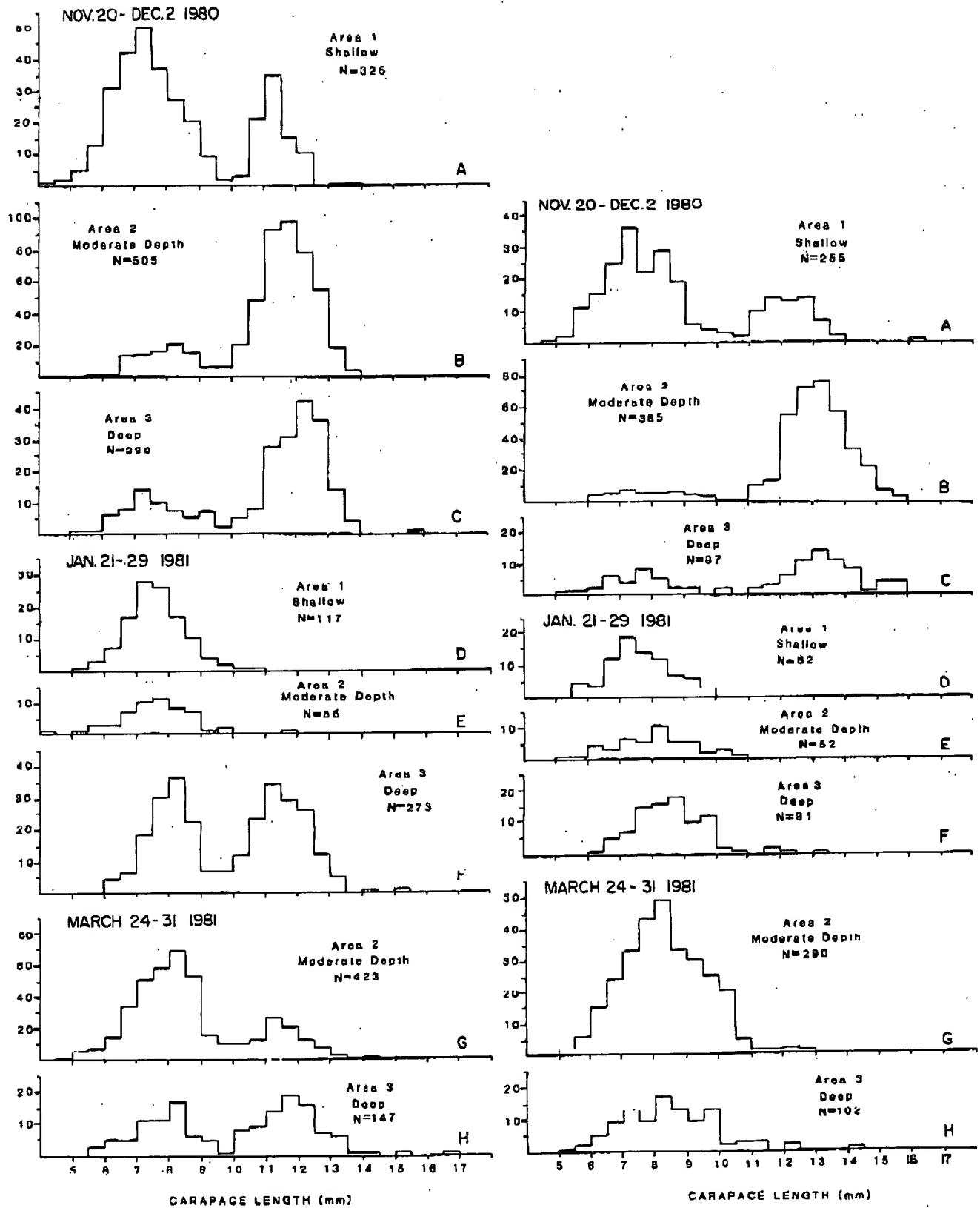


FIGURE 4.—Male *Dichelopandatus leptocerus* numerical length-frequency distributions by area and depth range.

however, may still have been a result of mortality rather than migration. The depth-area length-frequency data also revealed that slightly larger shrimp of both sexes and age-groups were captured in deeper water further down the Bay, suggesting that larger individuals are more likely to migrate than smaller ones.

Older male *P. montagui* also migrated down the Bay into deeper water during the winter: older age-1 males were more abundant than younger 0 age-group males in areas 2 and 3 (moderate and deep water, respectively) in November-December 1980 (Fig. 6A, B, C) and by late January nearly all the older males were in deep water in area 3 (Fig. 6D, E, F), but were not as abundant as the younger males in deep water in area 4 (Fig. 6G). There was no clear evidence that the larger males in either age-group

were more frequent in deeper water and not enough females were collected during any single sampling period to permit an analysis of differential distribution of different age-groups by depth.

Allen (1963) reported an offshore migration of age-1 males to deeper water in the spring and summer in Northumberland waters; Couture and Trudel (1969a) observed the same phenomenon in Grand-Rivière in the summer as the temperature exceeded 6°C and reported that it was triggered by the onset of maturity; earlier maturing males migrated sooner than later maturing males, indicating that migration was a function of size, not age. Mistakidis (1957) and Allen (1963) both reported an offshore migration of *P. montagui* females in the fall; Allen, however, reported that the smaller females stay behind to spawn in shallow water and that there was no "massive" return migration in the spring whereas Mistakidis reported a general offshore fall migration of females and a return migration in the spring. Allen (1963) reported that females in Northumberland which survived to spawn a third time were only found in depths > 100 m.

CONCLUSIONS

This study of the life histories of *Pandalus montagui* and *Dichelopandalus leptocerus* in Penobscot Bay has revealed some notable differences in reproductive characteristics and (apparently) in longevity between the two species. On the other hand, growth rates and migratory behavior were similar.

Most importantly, *P. montagui* is hermaphroditic; *D. leptocerus* is not. In 1980-81, some transitional *P. montagui* were observed during all five sampling periods, but were most common in the early spring; most individuals change sex at the end of their second year but some do so during their first year and a few may not change sex until their third year. Some individuals apparently begin life as females; 15% of the 0 age-group which recruited to trawl catches at 5-10 mm CL in November-December 1980 were females. Ovigerous *P. montagui* were collected over a longer time period, owing, at least in part, to the presence of more age-groups in the spawning population. Most *D. leptocerus* females spawn during their second year, although a few also spawn in their first year; ovigerous females were collected primarily in late November-early December. *Pandalus montagui* spend 1-2 yr as males; individuals which undergo sex transition in their second year may function as females during their second, third, and fourth years while those which change sex in their third year may only function as females for 2 yr. Judging from the

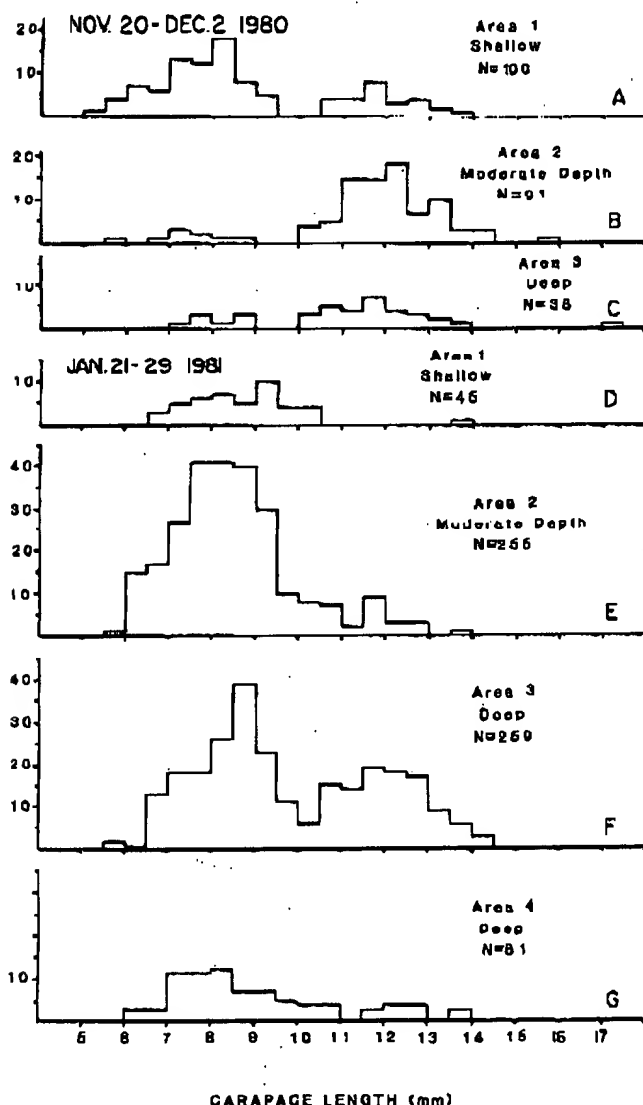


FIGURE 6.—Male *Pandalus montagui* numerical length-frequency distributions by area and depth range.

length-frequency data collected during this study, it is unlikely that very many *P. montagui* survive in the Bay beyond age 4. No *D. leptocerus* females older than age 2 were caught in the Bay although some males remained until their third fall (age, 2 yr and 9 mo). Conclusions concerning longevity were complicated by the fact that larger individuals of both species migrated down the Bay into deeper water as the winter progressed and were not captured and by the difficulty of inferring age from length data for the larger size-groups, particularly for *P. montagui*.

Both species were similar with respect to growth and migratory behavior. Growth decreased with increasing age and was seasonal, i.e., rapid in the spring and summer of the first year and the spring of the second year and negligible in the fall and winter. Males of both species reached 7.9 mm CL at age 1 and 11.13 mm CL at age 2. The data suggested that early (age 1) transitional *P. montagui* also grow considerably more rapidly than the remainder of their age group which undergo sex transition at age 2. The slowest observed growth rate was for *P. montagui* which apparently begin life as females and therefore never undergo sex transition. For *D. leptocerus*, females grow more rapidly than males and differential growth is a "fixed" sexual attribute. For *P. montagui*, the fact that the faster growing individuals change sex a year earlier and therefore function as females for an additional year (assuming that fast- and slow-growing shrimp have identical lifespans) means that more rapid growth and early sex transition increase the reproductive potential of the population, as long as enough males remain in the population to mate with the females. Female maturity is clearly a function of size, not age.

The life cycle of *P. montagui* in Penobscot Bay was quite different in several respects from the life cycles of populations which have been studied in the North Sea and at Grand-Rivière, Quebec. *Pandalus montagui* populations in two locations in the North Sea (Mistakidis 1957; Allen 1963) appeared to grow more rapidly in their first year of life than in Penobscot Bay and were composed of a considerably greater proportion of early maturing females, many of which never functioned as males. Growth over the entire lifespan was considerably more rapid in Penobscot Bay and the North Sea than at Grand-Rivière (Couture and Trudel 1969b), but the relative scarcity of females which do not function first as males and the delay of most sex transition until the third year were common to the Canadian and United States populations. In addition to a difference in the timing of maximum sex transition from age 2 (Penobscot Bay and Grand-Rivière) to age 1 (North Sea), the

seasonal intensity of sex transition was not the same in three of the populations. Most transitionals were observed in March in Penobscot Bay, in June in Northumberland (Allen 1963), and in October at Grand-Rivière. (Couture and Trudel 1969b). Eggs were carried by females in both North Sea locations and in Penobscot Bay during the winter (November-March); at Grand-Rivière most ovigerous females were observed in October.

In Penobscot Bay and the North Sea, sex transition tended to follow the end of the breeding season, whereas in Grand-Rivière, maximum sex transition coincided with the time when most females were carrying eggs (unless sex transition was more common later in the fall when no samples were collected), suggesting that there was a 12-mo interval between the appearance of external female characteristics and spawning at Grand-Rivière, and a 6-9 mo interval in Penobscot Bay and Northumberland. More rapid growth rates in the latter two locations would explain the shorter time intervals between sex transition and spawning. The reproductive cycle in Grand-Rivière was seemingly continuous, beginning in July and ending in June (Couture and Trudel 1969b).

Although an offshore migration of larger male *P. montagui* was observed in Penobscot Bay, Grand-Rivière, and Northumberland, this migration occurred in the winter following the end of the spawning season in the Bay and in spring and summer, prior to spawning, in the other two locations. Similar movements of larger females have been noted in both North Sea populations in the fall. Unlike the other migrations, the one observed in Penobscot Bay was not a spawning migration and may instead have been a response of older shrimp to declining winter temperatures in the shallower waters of the upper Bay. The departure of significant numbers of older shrimp from the Bay could certainly affect any conclusions concerning the size or age structure of either population and their estimated maximum lifespans.

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